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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

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Some Enzymatic Properties of Brain Acetylcholinesterase from Bluegill and Channel Catfish[1,2]

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ABSTRACT

Using a manometric technique an acetylcholinesterase (EC 3.1.1.7, acetylcholine acetylhydrolase) was demonstrated in brain tissue from the bluegill, *Lepomis macrochirus* Rafinesque, and the channel catfish, *Ictalurus punctatus* (Walbaum). The activities were 19 and 37 μmoles acetylcholine hydrolyzed/milligram protein per hour for the bluegill and channel catfish enzymes, respectively. The optimum substrate concentration for the hydrolysis of acetylcholine was 10 mM for the enzymes from both species. Generally, the catfish acetylcholinesterase was somewhat more susceptible than the bluegill to the inhibitors tested; however, the bluegill enzyme was more susceptible to inhibition by malathion and malaoxon.

INTRODUCTION

The increasing use of organophosphorus insecticides is causing widespread concern regarding their potential detrimental effect on the aquatic ecological system. These compounds usually enter the aquatic environment either indirectly through accidental contamination during or after agricultural applications, or directly through applications designed to control aquatic insect pests.

Organophosphorus insecticides are unusual in that we know their most probable mode of action in both vertebrate and invertebrate animals. In vivo and in vitro inhibition of acetylcholinesterase (AChE, EC 3.1.1.7 acetylcholine acetyl-hydrolase) by organophosphorus compounds have been reported in a variety of tissues from a wide range of animals (Heath, 1961).

In the past, organophosphorus insecticides were thought to degrade rapidly and were not considered a chronic hazard in the aquatic environment. Recently, however, it was shown that under certain conditions some of these compounds retained anticholinesterase activity for a year or more while in aqueous solution (Weiss and Gakstatter, 1965).

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Inhibition of fish brain AChE by organophosphorus compounds has been reported in vivo (Weiss, 1958, 1959, 1961; Williams and Sova, 1966) and in vitro (Weiss and Gakstatter, 1965). Also, both freshwater fish (Weiss, 1958, 1959, 1961; Hazeltine, 1963; Weiss and Gakstatter, 1964, 1965) and marine fish (Williams and Sova, 1966; Holland et al., 1967) have been used as biological monitors of organophosphorus insecticide contamination.

Properties of brain AChE in two species of marine fish were reported by Baslow and Nigrelli (1964) and Abou-Donia and Menzel (1967). Numerous investigators have used biochemical techniques to study certain of the properties of brain AChE from freshwater fish (Nachmansohn et al., 1941; Mendel and Rudney, 1943; Lindeman, 1945; Weiss, 1958, 1959, 1961; Baslow and Nigrelli, 1961, 1964; Weiss and Gakstatter, 1964, 1965; Verzhinskaya and Leibson, 1966). However, only one detailed study has been conducted: Lüdtke and Ohnesorge (1966) investigated the nature of the cholinester-hydrolysing enzyme from the brain of tench, *Tinca tinca*.

Before a fish or any other organism can be used to assess at the biochemical level the magnitude of environmental contamination resulting from an AChE-inhibiting insecticide, certain data should be obtained regarding the nature of the enzyme. For example, it should be shown in studies conducted in vitro that the AChE is the only enzyme hydrolyzing the substrate selected for the assay. In addition, the assay conditions should be as close to optimum as possible if the data are to reflect the inhibition level in vivo. The purpose of the present study, therefore, is to determine some of the enzymatic properties of brain AChE from bluegill, *Lepomis macrochirus* Rafinesque, and channel catfish, *Ictalurus punctatus* (Walbaum), so that these organisms may be used as biological monitors of organophosphorus insecticides.

MATERIALS AND METHODS

Enzyme Preparation

Bluegills were obtained from Corning National Fish Hatchery, Corning, Arkansas, and were 60–100 mm long (average weight 9 g). Channel catfish were from the Fish Farming Experimental Station, Stuttgart, Arkansas, and were 87–100 mm long (average weight 7 g).

The brains were excised, wet-weighed, and homogenized immediately in bicarbonate buffer (0.0357 M sodium bocarbonate and 0.164 M sodium chloride, gassed with 95% N_2–5% CO_2 to *p*H 7.6) at 5 C in a glass homogenizer fitted with a Teflon® pestle. With the exception of the storage experiments discussed below the homogenate was used immediately after preparation.

Protein was determined by the Miller (1959) modification of the Folin phenol method (Lowry et al., 1951); fresh bovine serum albumin (Nutritional Biochemical Corp., Cleveland, Ohio) was used as the standard.

Substrates and Inhibitors

Esters tested as enzymic substrates included acetylcholine iodide (ACh), propionylcholine-*p*-toluenesulfonate (PrCh), butyrylcholine-*p*-toluenesulfonate

(BuCh), acetyl-β-methylcholine bromide (MeCh), benzoylcholine chloride (BzCh), phenyl acetate, phenyl-*n*-butyrate, glyceryl triacetate, glyceryl tri-*n*-butyrate, and methyl-*n*-butyrate.

The followings compounds were used as inhibitors: 2,2-dichlorovinyl dimethyl phosphate (dichlorvos), from Shell Development Co., Modesto, Calif.; diethyl *p*-nitrophenyl phosphate (paraoxon) from K and K Laboratories, Inc., Plainview, N.Y.; di*iso*propyl phosphorofluoridate (DFP) from Aldrich Chemical Co., Inc., Milwaukee, Wisc.; diethyl mercaptosuccinate *S*-(*O*,*O*-dimethyl phosphorodithioate) (malathion) and diethyl mercaptosuccinate *S*-(*O*,*O*-dimethyl phosphorothiolate (malaoxon) from American Cyanamid Co., Princeton, N.J.; 1,5-*bis* (4-trimethylammonium phenyl) pentan-3-one diiodide (62C47) from Burroughs Wellcome and Co., Inc., Tuckahoe, N.Y.; and eserine sulfate from Nutritional Biochemical Corp., Cleveland, Ohio.

Esterase Assay Method

The manometric method was used to estimate the esterase activity, and the procedure was identical to that employed by Knowles and Casida (1966).

Esterase activity was studied as a function of substrate concentration with the choline esters. The abbreviation pS is used to indicate the negative logarithm of the molar substrate concentration. The noncholine esters were used at 10mM. Those which were not water-soluble at this level were emulsified in the buffer with Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.).

Activity is expressed as μmoles substrate hydrolyzed per mg protein per hour. Data are means from at least duplicate determinations in separate runs.

Inhibition of Esterase Activity

The procedure for studying the inhibition of esterase activity has been described (Knowles and Casida, 1966). Inhibitory data are expressed as pI_{50}, the negative logarithm of the molar inhibitor concentration for 50% inhibition, or as percentage inhibition at a given molar inhibitor concentration.

RESULTS

The rate of hydrolysis of ACh in both bluegill and channel catfish was proportional to the concentration of enzyme in the homogenate (Fig. 1). Aliquots of a 0.5% homogenate (bluegill) and a 0.3% homogenate (catfish), amounts approximately equivalent to 0.5 and 0.3 mg protein/flask, were used for subsequent studies. When either whole brains or homogenates of brains of the bluegill were stored at −20 C, the AChE activity varied as much as 10% over a 4-week period.

AChE's from both fish displayed typical bell-shaped activity-substrate concentration curves (Fig. 2). The ACh concentration for optimum activity was 10 mM for both the bluegill and catfish enzymes. However, the catfish AChE was almost twice as active as the bluegill enzyme when assayed at the optimum

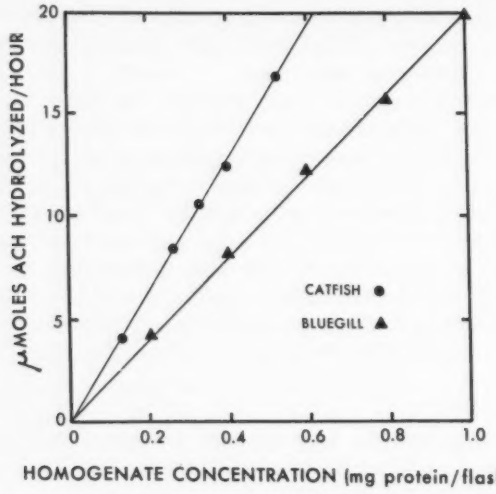

FIG. 1. Hydrolysis of acetylcholine (10 mM) by fish brain acetylcholinesterase as a function of homogenate concentration.

ACh concentration. MeCh was hydrolyzed by the bluegill AChE and had the same substrate optimum as the choline esters. ACh was hydrolyzed at a faster rate than was MeCh at concentrations of 10 mM. Neither BuCh nor BzCh were hydrolyzed appreciably by either AChE.

The carbamate eserine was a potent inhibitor of both AChE's; the pI_{50} values were 6.6. and 6.7 for the bluegill and catfish, respectively (Table I). The potency of the selective AChE inhibitor 62C47 was in the same order of magnitude as that reported for AChE's from other sources (Knowles and Casida, 1966; Bratkowski and Knowles, 1968). The organophosphates varied considerably in their inhibition of the two AChE's and, except for malathion and malaoxon, the catfish enzyme was considerably more sensitive. As was anticipated, malaoxon, the phosphorothiolate analog of malathion and the toxic anticholinesterase metabolite of malathion in vivo, was more potent (>400 times) than malathion to both AChE's.

Attempts to isolate the bluegill AChE in a discrete centrifugal fraction were only moderately successful. For example, when a brain homogenate prepared in the bicarbonate buffer was centrifuged at 32,700 *g* for 60 min at 5 C, about 70% of the AChE activity remained in the particulate fraction.

Since AChE's are known to be affected by certain divalent cations, we tested their action on the fish enzymes (Table II). In these studies the sodium chloride was excluded from the buffer. Mg^{2+} and Mn^{2+} activated both bluegill and catfish AChE's whereas Cu^{2+} and Ni^{2+} were inhibitory to both enzymes. Co^{2+} inhibited the bluegill enzyme only.

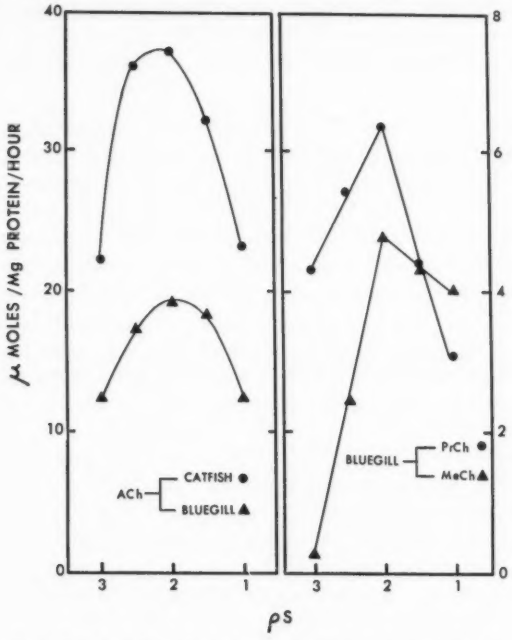

FIG. 2. Activity-substrate concentration curves for the hydrolysis of acetylcholine, propionylcholine, and acetyl-β-methylcholine by fish brain acetylcholinesterase. pS, negative logarithm of molar substrate concentration.

TABLE I. Inhibition of fish brain acetylcholinesterase by certain compounds.

	Bluegill		Channel catfish	
Compound[a]	pI_{50}[b]	Slope[c]	pI_{50}	Slope
Eserine	6.6	0.61	6.7	0.49
62C47	5.2	0.80	5.4	1.05
DFP	4.4	1.25	6.1	0.91
Dichlorvos	5.6	1.05	6.3	1.25
Paraoxon	5.8	1.11	6.7	1.25
Malathion	4.2	1.11	3.5	0.77
Malaoxon	6.8	1.05	6.2	0.53

[a]Inhibitors were preincubated with enzyme for 40 min prior to the addition of acetylcholine.
[b]Negative logarithm of the molar inhibitor concentration required for 50% inhibition of the enzyme.
[c]Calculated as $\frac{1}{pI_{20}-pI_{80}}$, where pI_{20} and pI_{80} are the negative logarithms of the molar concentrations of the compounds necessary to produce 20 and 80% inhibition of the acetylcholinesterase.

TABLE II. Effect of divalent cations on fish acetylcholinesterase activity.

Cation[a]	% activity	
	Bluegill	Channel catfish
Ca	104	105
Co	85	105
Cu	2	0
Mg	116	127
Mn	120	120
Ni	14	66

[a]Cations at concentrations of 1 mM were preincubated with enzyme for 40 min prior to the addition of acetylcholine.

Several noncholine esters were also hydrolyzed by enzymes present in the brain homogenates (Table III). Phenyl acetate was hydrolyzed at the fastest rate and glyceryl tri-*n*-butyrate at the slowest rate by both brain homogenates. A considerable portion of the hydrolytic activity for glyceryl triacetate and phenyl acetate was inhibited by eserine, indicating the probable participation of the AChE in the hydrolysis of these substrates. These data indicate also the presence of esterases other than the AChE; however, attempts to separate these esterases from the AChE by differential centrifugation were unsuccessful.

TABLE III. Substrate and inhibitor specificities of bluegill (B.G.) and channel catfish (C.C.) brain esterases.

Substrate[a]	Activity[b]		% inhibition			
			Eserine, 10^{-4} M		Dichlorvos, 10^{-5} M	
	B.G.	C.C.	B.G.	C.C.	B.G.	C.C.
Acetylcholine	19.1	37.1	100	100	87	100
Glyceryl triacetate	3.8	3.5	61	38	62	68
Glyceryl tri-*n*-butyrate	1.4	2.3	–	–	–	–
Phenyl acetate	16.0	37.7	74	82	83	92
Phenyl butyrate	3.9	4.7	9	4	63	52

[a]Acetylcholine concentration was 10 mM; other substrates were used as emulsions. Methyl-*n*-butyrate was not appreciably hydrolyzed.
[b]μmoles/milligram protein per hour.

DISCUSSION

The cholinester-hydrolyzing enzymes present in brain homogenates of bluegills and channel catfish displayed the characteristics of an AChE. AChE's are inhibited by low concentrations of eserine and by excess substrate. They

hydrolyze ACh faster than BuCh at optimum substrate concentrations, and they hydrolyze MeCh but not BzCh (Heath, 1961). Also, the fish enzymes are inhibited by 62C47, a selective AChE inhibitor (Todrick, 1954; Fulton and Mogey, 1954).

Specific activities for the hydrolysis of ACh were 19 and 37 μmoles/mg protein per hour for the bluegill and channel catfish, respectively. When calculated on the average brain size (46 mg for the bluegill and 77 mg for the channel catfish and assayed in the presence of 1 mM Mg^{2+}, the activity values were 1.5 and 2.6 μmoles/mg brain (wet weight) per hour for the bluegill and channel catfish, respectively. Weiss (1959, 1961), using a phosphate buffer containing 20 mM Mg^{2+} and the Hestrin technique, reported the following activities for fish brains of comparable size (~45 mg): bluegill, 1.8; largemouth bass, *Micropterus salmoides*, 2.1; golden shiner, *Notemigonus crysoleucas*, 2.3; and goldfish, *Carassius auratus*, 2.2. The AChE preparation from channel catfish brain in the present study was more active than any of the fish preparations listed above.

The optimum substrate concentration for the hydrolysis of the choline esters and MeCh was 10 mM for both bluegill and channel catfish brain AChE's. Utilizing tench brain AChE, Lüdtke and Ohnesorge (1966) observed a bell-shaped activity-pS relationship with maximum hydrolysis of ACh occurring at a concentration of 4 mM.

In the inhibition studies the channel catfish AChE was approximately five times less sensitive to malathion and malaoxon than was the bluegill enzyme; however, the channel catfish AChE was more sensitive to the other inhibitors employed in the study. The channel catfish also is less susceptible than the bluegill to poisoning by malathion in vivo (O. B. Cope, personal communication). Weiss and Gakstatter (1965) studied the inhibition of goldfish AChE in vitro by 11 organophosphorus insecticides. The only compounds common to both studies are dichlorvos (DDVP) and malathion. Expressing the data of Weiss and Gakstatter (1965) in terms of pI_{50} values, we calculated figures of 4.6 for dichlorvos and 3.1 for malathion. This apparent relative insensitivity of goldfish AChE to dichlorvos is unusual. In the present study the pI_{50} values for dichlorvos were 5.6 and 6.3 and those for malathion were 4.2 and 3.5 for the bluegill and catfish AChE's, respectively. Turning to invertebrates, Bratkowski and Knowles (1968) reported that the pI_{50} value for dichlorvos and head AChE of the house fly, *Musca domestica*, was 9.4; for dichlorvos and head AChE of the face fly, *M. autumnalis*, 9.2. However, the pI_{50} value of 6.6 obtained by Knowles and Casida (1966) for AChE from the swine helminth, *Ascaris lumbricoides*, for dichlorvos inhibition is closer to the values obtained for bluegill and channel catfish.

It is difficult to determine the number of esterases present in the fish brain homogenate from the data presented. Several esterolytic enzymes appeared to be present. However, the substrate specificity and inhibitor sensitivity data did indicate clearly that only the AChE was hydrolyzing ACh.

The fact that the AChE from both fish appeared in more than one centrifugal fraction is not necessarily evidence for the existence of more than one AChE. Smallman and Wolfe (1956) found that the AChE from the house fly head existed in both a soluble and a particulate form, and the distribution was influenced by the concentration of salt present.

The primary aim of this work was to study the properties of the fish AChE under conditions similar to those that will be employed for analysis of fish AChE inhibited by organophosphates in vivo. Hence, no further attempts were made to purify the AChE. An elaborate purification scheme would not be feasible with a large number of samples. Moreover, phosphorylated-AChE's are reactivated by nucleophilic reagents (Heath, 1961), and any extensive purification techniques would make the inhibitory data suspect.

These in vitro studies have described certain of the properties of the brain AChE from bluegill and the channel catfish. We utilized a technique that is both convenient and feasible for determining in vivo AChE inhibition in fish resulting from exposure to organophosphorus insecticides.

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Relation Between Production and Mineralization of Organic Matter in Aburatsubo Inlet, Japan[1,2]

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ABSTRACT

Studies carried out in Aburatsubo Inlet (35°09′N, 139°37′E) showed that generally during the spring the dark assimilation of carbon dioxide was a large fraction of the total carbon dioxide assimilated. During the rest of the year photosynthesis accounted for most of the production of the water column, but dark assimilation was sometimes appreciable in the sediments. Production generally exceeded mineralization, indicating a net gain of organic matter either in the sediments or in adjoining water masses.

INTRODUCTION

Marine microorganisms are chiefly responsible for the link between production and mineralization of organic matter in the sea. Moreover, as the two processes depend upon each other, they should be examined simultaneously when the dynamics of biological substances in the sea are studied. These processes can be simply expressed as uptake and release of carbon dioxide by the microorganisms: production of organic matter is simply indicated by microbial uptake of carbon dioxide, and mineralization by release, although some fraction of produced organic matter is dissolved into the sea (Guillard and Wangersky, 1958) and may be resynthesized from the soluble to the particulate form by heterotrophic organisms. The production of organic matter in the seas and oceans has been well investigated by the carbon-14 method since the method was introduced by Steemann Nielsen (1952). On the other hand, little information is available on the mineralization of organic matter in the sea.

A study was designed, therefore, to investigate the link between production and mineralization of organic matter in a coastal region and to analyze its significance in the exchange of biological substances in the seas and oceans. Investigations were conducted throughout the year, with an average sampling interval of about 10 days, at a fixed station in Aburatsubo Inlet, Japan, and data representative of seasonal changes in mineralization and production are reported and discussed in the following presentation.

MATERIALS AND METHODS

Seawater samples were collected aseptically with Hyroht sampling bottles. All the procedures for culturing were done within half an hour after the collection of samples, employing aseptic techniques.

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[2]Contribution from Japanese International Biological Program (JIBP-PM).
[3]Present address: Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

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The rate of microbial uptake of carbon dioxide in the water was measured in situ. $Na_2{}^{14}CO_3$ solution (1 ml) with an activity of 2 μ Ci was added to 99 ml of sea water in a sample bottle. The samples were incubated from 11:30 AM to sunset. Samples kept in the presence of 10% neutral formaldehyde were employed as controls, and treated in the same way as the experimental samples. After the incubation, the experimental and control samples were filtered through HA Millipore filters (pore size: 0.45 μ). The filters were washed with 3% NaCl solution, then with 0.1% HCl in 3% NaCl solution, and again with 3% NaCl solution to remove $Na_2{}^{14}CO_3$. After complete drying, the radioactivity of the samples was determined with a 2 π gas-flow counter (Aloka Radiation Counter).

One gram of the topmost layer of marine sediments was collected with a Phleger corer for the determination of the microbial uptake in sediments. Self-absorption correction for β radiation in the sediment samples was determined by the method of Seki and ZoBell (1967).

Glucose uptake by microorganisms in seawater samples was measured according to the method of Parsons and Strickland (1962) and an incubation time of 6 hr was employed for all experiments. D-Glucose-^{14}C (u) solution (1 ml) with an activity of about 2 μ Ci was added to each sample of 99 ml. Carrier glucose was also added, to a final concentration of 0.4 mg C per litre; it will be shown later that the amount added was sufficiently large in relation to the total glucose concentration usually encountered in the inlet.

The rate of mineralization of organic matter in sea water was measured by determining the release of carbon dioxide in the dark (Seki, 1967). Two litres of natural sea water were filtered aseptically through a HA Millipore filter. The filter, on which microorganisms and organic matter were retained, was then crushed into a 30-ml sterilized glass bottle in which 10 ml sterilized seawater medium had been completely aerated by shaking previously. The medium was then sealed with sterilized liquid paraffin, through which the samples for analysis were collected aseptically. The liberation of carbon dioxide into the medium was assumed to reflect mineralization and was measured manometrically. A check by the BOD method showed that the action of filtering did not appreciably decrease microbial activity. Incubation was carried out at in situ temperatures for less than 6 hr.

Carbon dioxide in seawater samples was analyzed by a modified Van Slyke method with a Yufu micro-blood–gas-analyzer (Saito, 1957). Light intensity was measured by a 2 π submarine photometer (Toshiba No. 9). The *p*H and redox potential of sea water were measured by *p*H meter model DM-IA (Toa Electronics Ltd.) and redox meter model RM-I (Toa Electronics Ltd.) respectively. Total carbohydrates in sea water were measured by the phenol sulphuric acid method (Handa, 1966).

Heterotrophic bacteria are only a part of the biochemical agents responsible for the mineralization of organic matter in the sea. Nevertheless, as they can be detected on any particulate matter in the inlet, heterotrophic bacteria, as enumerated by cultural methods, may indicate the degree of

mineralization. The relationship between mineralization and number of heterotrophic bacteria was studied throughout the year. Heterotrophic bacteria were enumerated by the agar poured-plate method and incubations were carried out at 20 C for 10 days. The plating medium (modified medium 2216) had the following composition: polypeptone (Daigo Eiyo Kagaku Co.), 0.5%; ferric phosphate soluble, 0.001%; agar, 1.5%; aged sea water, final *p*H 7.8. In this case, as heterotrophic bacteria were enumerated by the agar poured-plate method, the numbers of bacteria indicated were actually the numbers of bacterial clumps which were derived from bacterial populations on detrital particles.

RESULTS

Seasonal Fluctuation

Vertical differences in the production and mineralization of organic matter were investigated throughout the year in the inlet and representative results for 4 days in the year are shown in Fig. 1–4, together with some environmental factors affecting the microbial activity.

On September 23, which was representative of both summer and autumn, photosynthesis accounted for most of the carbon fixation in the water column except at the mud surface (Fig. 1). When the measurements were taken, the temperature of the water column was about 22 C and about 8% of the incident radiation penetrated to the bottom. Under these conditions the predominant form of carbon fixation in the water column was by photosynthesis, except at the mud surface where there was an immeasurably small amount of photosynthesis, but a large chemosynthesis and heterotrophic assimilation. Photosynthesis exceeded mineralization in the water column, but at the mud surface mineralization was greater than chemosynthesis and heterotrophic assimilation.

On February 25, which was representative of the winter period, photosynthesis was appreciable at the mud surface but production was less than mineralization throughout the water column except at the sea surface (Fig. 2). Uptake of carbon dioxide by chemosynthesis and heterotrophic assimilation was low in the water column but high at the mud surface. The temperature of the water column was about 13 C and more than 20% of the incident radiation penetrated to the mud surface.

On April 25, which was representative of the spring, the quantity of chemosynthesis and heterotrophic assimilation was comparable with that of photosynthetic assimilation both in the water column and at the mud surface (Fig. 3). At this surface the dark assimilation of carbon dioxide exceeded photosynthesis. During this season it was also observed that heterotrophic bacteria increased in the water column and decreased in the mud by one or two orders of magnitude, i.e. around 10^4 heterotrophic bacteria per millilitre were enumerated in the water column in this month compared with 10^2–10^3 per millilitre during other times of the year.

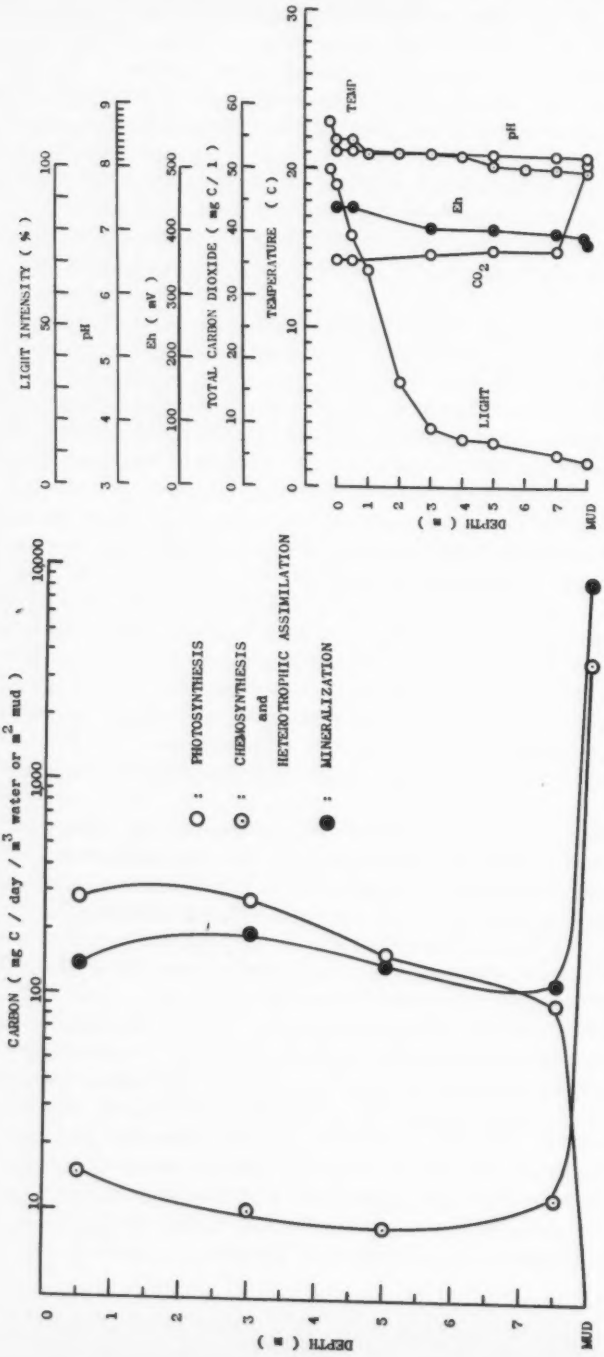

FIG. 1. Production and mineralization of organic carbon on September 23, 1966.

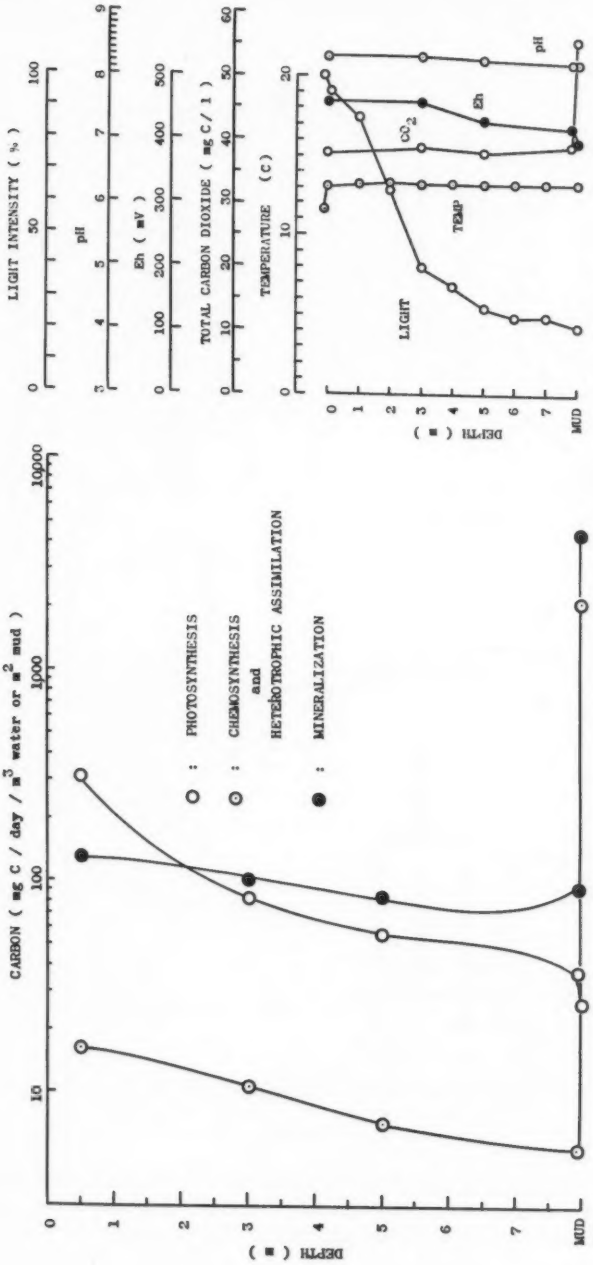

FIG. 2. Production and mineralization of organic carbon on February 25, 1967.

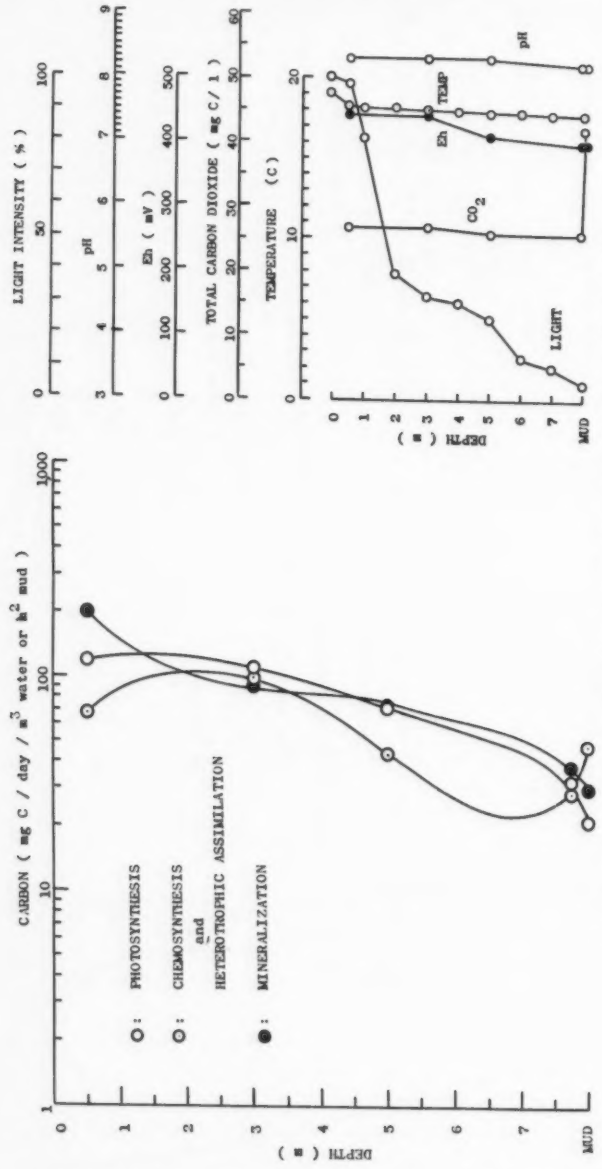

FIG. 3. Production and mineralization of organic matter on April 25, 1967.

On May 26 (Fig. 4), which was representative of the transitional period between spring and summer, conditions of organic exchange were similar to those in autumn (Fig. 1) with the exception that appreciable photosynthesis occurred at the mud surface.

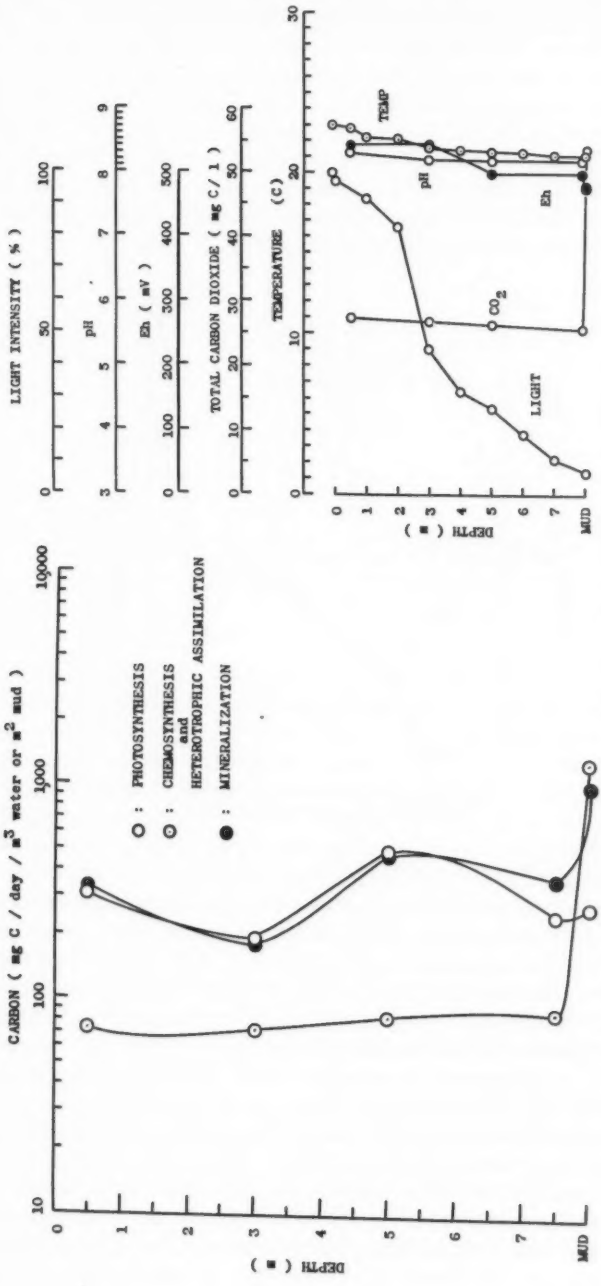

FIG. 4. Production and mineralization of organic matter on May 26, 1967.

The seasonal quantitative balance of organic matter per metre^2 of the water column is summarized in Fig. 5. Production generally exceeded mineralization in the water column of the inlet. An early summer maximum was observed in both production and mineralization; the chlorinity of the sea water was then 18.5‰, indicating that the water of the inlet was largely influenced by the lower salinity water of Tokyo Bay. When a winter minimum in both production and mineralization was observed, the chlorinity of the sea water was about 19‰ suggesting that a considerable intrusion of water from the Kuroshio current existed in the inlet.

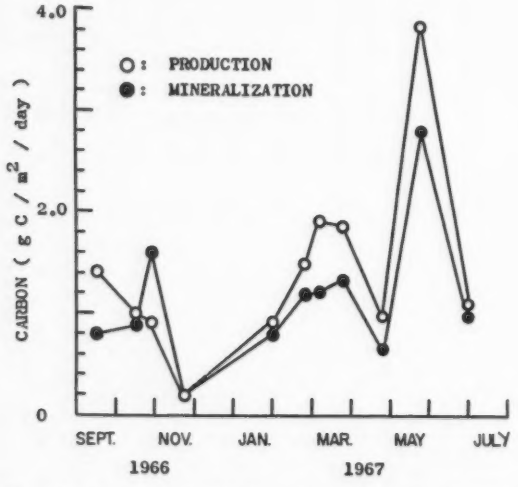

FIG. 5. Seasonal fluctuation of production and mineralization in Aburatsubo Inlet.

SIGNIFICANCE OF DARK ASSIMILATION

In all seasons except spring, dark assimilation of carbon dioxide was only a few per cent of assimilation by photosynthesis and dark assimilation was generally more pronounced in the deeper parts of the inlet, where percentages increased up to about 20% at the bottom (Fig. 6). On the other hand, during the spring, dark assimilation of carbon dioxide was at times greater than 50% of photosynthesis at all depths of the water column.

MINERALIZATION OF ORGANIC MATTER

The rate of mineralization per clump of heterotrophic bacteria decreased as concentrations of heterotrophic bacteria increased in the water column (Fig. 7). The common logarithm of mineralization per clump versus the common logarithm of the heterotrophic bacteria fits a linear relationship in both Aburatsubo Inlet and Sagami Bay. The two slopes are quite different, however, which may be attributed to a difference in species.

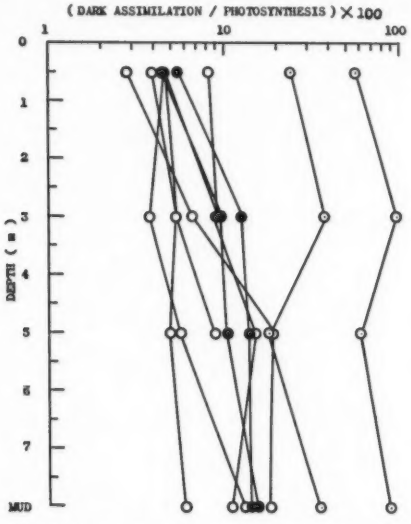

FIG. 6. Dark assimilation of carbon dioxide as a per cent of photosynthesis. (Percentages for observations made during: ⊙ = spring; ○ = summer and autumn; ● = winter.)

The distribution of organic matter has been shown to be one of the most important factors influencing microzonation in the distribution of heterotrophic bacteria in Aburatsubo Inlet (Seki, 1966). In order to evaluate the nutritive potential of the suspended organic matter, the mineralization of this material within a 10-hr period was compared with that in a medium containing 1% polypeptone. This is reported in Fig. 8 as the *heterotrophic index*, which was determined as the percentage mineralization under optimum nutrient conditions versus that obtained from natural material retained on a filter. Heterotrophic indices of about 200% were observed in the inlet throughout the year. On the other hand, much higher values were measured in the oceanic region of Sagami Bay, which can be attributed to the higher activity of microorganisms or more scarce nutrient supply in the oceanic region.

Relationships between mineralization activity and glucose uptake were also investigated in the inlet, as both processes are heterotrophic processes of microorganisms in the sea. Total amounts of carbohydrate in sea water, expressed as D-glucose equivalents, were around 0.5 mg per litre in winter and around 2.0 mg in summer and autumn. Dissolved carbohydrate amounts in sea water were less than 0.5 mg per litre throughout the year. The quantity of carrier glucose added, therefore, was sufficient to derive a relative measure of heterotrophic activity. As shown in Fig. 9, 0.2–1.0 mg C glucose per m^3 per hour was assimilated, and there was an inverse relation between mineralization and glucose uptake.

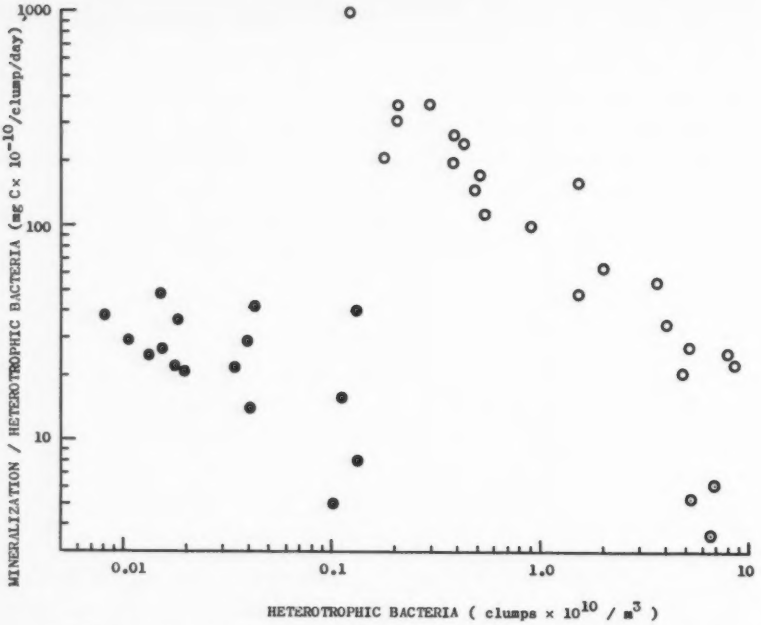

FIG. 7. Relationship between mineralization rate and concentration of clumps of heterotrophic bacteria in sea water, measured by plate counts. Data from: ● = Kuroshio current in Sagami Bay (Seki, unpublished data); ○ = Aburatsubo Inlet; ⊙ = *Zostera* zone in Aburatsubo Inlet.

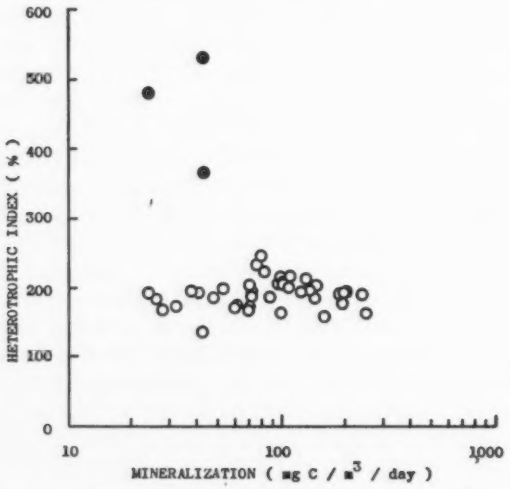

FIG. 8. Effect of organic nutrient on natural microorganisms in sea water. Data from: ● = Sagami Bay (Seki, unpublished data); ○ = Aburatsubo Inlet.

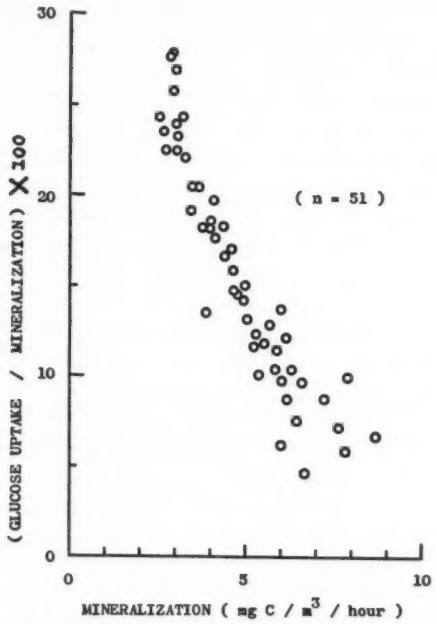

FIG. 9. Glucose uptake as a per cent of mineralization.

DISCUSSION

Three types of seasonal fluctuations were observed in Aburatsubo Inlet. These occurred during the spring, summer and autumn, and winter. During the summer, autumn, and winter large amounts of carbon dioxide were assimilated in the dark by microorganisms in the sediments but relatively little or no photosynthesis was detected during the autumn and summer in the sediments. During the summer, autumn, and winter, only small amounts of organic matter were produced by dark assimilation in the water column. In the spring, dark assimilation of carbon dioxide was higher in the water column and lower in the sediments than at the other times of the year. This latter phenomenon may be chiefly attributed to the stirring up of the benthic microorganisms into the water column by the strong seasonal winds, although the influence of water coming from Tokyo Bay may also contribute to this phenomenon. During this season, dark assimilation of carbon dioxide was quantitatively very important in the exchange of biological substances in the sea.

Fluctuations in production and mineralization were generally simultaneous, i.e. mineralization increased or decreased with production. During most of the year organic matter was produced in excess of mineralization so that a certain fraction of organic material must have been transported to the

oceanic regions or deposited in bottom sediments. In the oceanic region of Sagami Bay off Aburatsubo Inlet, mineralization activity has often been found to exceed productivity even in the euphotic zone (Seki, unpublished data). This phenomenon indicated that allochthonous organic matter is transported from the coastal to the oceanic regions. In Aburatsubo Inlet, mineralization exceeded production only in the sediments as a rule.

The rate of mineralization per clump of heterotrophic bacteria was also examined. The decrease in rate of mineralization as the concentration of clumps of heterotrophic bacteria in the water column increased (Fig. 7) suggests that the mineralization activity of microbial clumps has an inverse relation to concentration of the clumps in sea water. The mineralization rate of the microorganisms was increased by the addition of organic nutrients. This effect was observed to be much more favorable for the microorganisms in the oceanic region of Sagami Bay off Aburatsubo Inlet than those in Aburatsubo Inlet, which indicated that the organic material in the latter environment was a better bacterial substrate than material from oceanic waters.

Glucose uptake by marine microorganisms is one of the heterotrophic processes in the sea and its relationship with mineralization activity was also investigated. The amounts of glucose assimilated corresponded to between 5 and 30% of the mineralization activity in Aburatsubo Inlet (Fig. 9). The rate of glucose uptake decreased when mineralization increased, giving an inverse relation throughout the year. This tendency may be largely due to the microbial availability of organic nutrients under various conditions.

In summary, it appears that the dark assimilation of carbon dioxide through either chemosynthetic or heterotrophic processes may be considerable in coastal areas during certain periods of the year, as illustrated here for Aburatsubo Inlet. Further, it appears that total production of organic matter in the water column may exceed mineralization for most of the year in a shallow coastal area. This must be compensated for by either the laying down of rich organic sediments or by the transportation of organic material out of the immediate environment by advective effects. The exact role of bacterial clumps in causing mineralization appears to bear an inverse relationship to their numbers, whereas heterotrophic activity appears to be directly related to either the number of microorganisms or the supply of nutrients.

ACKNOWLEDGMENTS

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Heavy Metal Ions and the Development of Rancidity in Blended Fish Muscle[1,2]

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ABSTRACT

From 1 to 50 ppm of 10 different heavy metal ions were added to blended muscle taken from freshly killed cod, haddock, flounders, redfish, herring, mackerel, scallops, and lobster, and stored for 24 hr at 0 C. The resulting rancidities were determined by thiobarbituric acid (TBA) values and odours. With some exceptions, Fe^{++}, V^{++}, and Cu^{++} were the most active catalysts. Fe^{++} was always more effective than Fe^{+++}. Cd^{++}, Co^{++}, and $Zn+$ produced randicity with the fatty species but not with any of the other species that were tested. Ni^{++}, Cr^{++}, Ce^{++}, and Mn^{++} did not accelerate rancidity in any of the muscles. There was considerable difference in the relative susceptibility of muscle from different species to rancidity induced by specific metals. During frozen storage, changes in fish muscle took place that rendered it less susceptible to subsequent metal-induced rancidities.

INTRODUCTION

Some heavy metal ions actively catalyze lipid oxidation and their presence, even in trace amounts, has long been recognized as potentially detrimental to the keeping time of fats, oils, and fatty foods. Less is known about the catalytic effect of these metal ions on lipids as they exist in muscle, particularly muscle with a relatively low fat content. One reason is that, in addition to reacting with lipids, some metals also form complexes and react with other compounds that are found in animal tissue. Lea (1952) has pointed out that in foods containing much nonfatty material a large and variable part of the heavy metal present is likely to be bound in inactive forms and unable to function as a catalyst. In such cases no correlation between the amount of copper or iron and keeping properties of the food can be demonstrated.

With this in mind, experiments have been carried out to determine the comparative effect of heavy metal ions on the development of oxidative rancidity in the muscle from a number of different species of commercial fish and other marine animals.

PROCEDURES AND MATERIALS

For most of the experiments, live fish were obtained from the station's aquaria. In a few instances (as with scallops), fresh samples were collected at sea in specially iced containers and the experiments were carried out within 24 hr after catching. The tests, therefore, were carried out on very fresh muscle in prerigor condition.

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[2]This is No. 9 in the series of papers dealing with rancidity in fish muscle.

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Muscle from the following species were used: Atlantic cod (*Gadus morhua*), Atlantic haddock (*Melanogrammus aeglefinus*), American plaice (*Hippoglossoides platessoides*), redfish (*Sebastes marinus*), Atlantic herring (*Clupea harengus*), scallop (*Placopecten magellanicus*), lobster (*Homarus americanus*), and oyster (*Crassostrea virginica*).

These particular species were selected from among the many that were available so as to include fish with a high fat content (herring and mackerel); semi-fat fish (ocean perch and flounder); lean fish (cod and haddock); a crustacean (lobster); and a shellfish (scallop). The shucked oysters do not belong in this series as the portion used was not confined to muscle. But as the processor and consumer deal with the whole animal rather than the muscle alone (as is the case of fish and scallops), it was decided to include the oyster as a matter of general interest. Some people are inclined to think that only fish with a high fat content are subject to oxidative rancidity. By including species with widely different fat content, it was hoped to show the importance of the total amount of fat in the muscle to the development of rancidity.

From some aspects it would have been preferable to work with whole muscle rather than blended muscle. It is realized that results obtained with tissue that has been disintegrated by grinding or blending may not be the same as those obtained with whole, unblended muscle or large chunks. Nevertheless for this work there were advantages to using a blend in place of whole muscle. The metal salt solutions could be more easily incorporated and more uniformly distributed. In the blend, lipid oxidation developed more uniformly and was more easily measured. Problems of handling and storage were simplified. And perhaps most important, by using similar weights of blended muscle, a more direct comparison could be made of the activity of the metals on muscle from different species of fish and animals.

The procedure was as follows. Freshly excised muscle was blended with three times its weight of distilled water for 45 sec in a Waring Blendor. In the case of fish, whole fillets were always used; with lobsters, only the tail muscle; with scallops, the adductor muscle; but with oysters the whole "shucked" meat was used as it came from the shell. From these blends 100-g amounts were distributed in 250-ml Erlenmyer flasks. For each experiment standard solutions were prepared from reagent grade salts (metal sulphates or chlorides). Particular care was taken to add the freshly prepared iron solutions to the blended muscle immediately, because of their tendency to precipitate if left standing. All other salt solutions were kept away from the light and used on the same day they were prepared. Dilutions of these solutions were added to the blended muscle so as to give the required concentrations. In every case the recorded concentrations refer to the parts per million (ppm) of metal ion added to the blend of muscle plus water. After mixing to distribute the salt solutions in the blend, the flasks were capped with aluminum foil and stored at 0 C. At 24 hr the extent of lipid oxidation was measured by the thiobarbituric acid (TBA) reaction and any off-odours that had developed were recorded. The TBA procedure used was that of

Tarladgis et al. (1960) with the modification suggested by Castell et al. (1966b) and Castell and Boyce (1966) to prevent metal-induced oxidation of the muscle lipids during distillation. This precaution is particularly important with blends to which iron or vanadium had been added and with all samples from fish, such as herring, that have relatively large amounts of dark muscle; it was also found necessary with the whole shucked oyster meats. The term "TBA values" is used to indicate the optical density reading at 538 mμ after reaction with the TBA reagent.

All the glassware used in these tests was sterilized, and care was taken to keep initial bacterial contamination in the muscle samples to a minimum. Frequent checks have shown that fresh, blended muscle, prepared with care and held at 0 C, will be free from microbial activity for periods beyond those used in these experiments (MacLean and Castell, 1964).

EXPERIMENTAL RESULTS

Relative activity of metal ions.— There was a considerable difference in the relative activity of the different metal ions in catalyzing lipid oxidation in the muscle (Fig. 1–9). They could be divided into three groups: (I) Fe^{++}, V^{++}, Cu^{+}, and Fe^{+++}, which produced the highest TBA values and strongly rancid odours with the lowest metal ion concentrations; (II) Cd^{++}, Co^{++}, and Zn^{++} formed a second group that were much less active. Even when added to give concentrations of 50 ppm these metals never produced rancid odours or high TBA values in any but the fatty fish; (III) In the concentrations used (1–50 ppm), Ni^{++}, Cr^{++}, Ce^{++}, and Mg^{++} had little or no effect on the development of rancidity in muscle from any of the species that were tested.

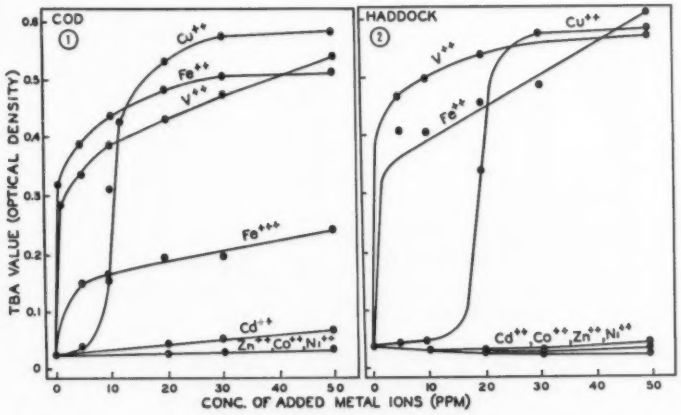

FIG. 1. Effect of adding heavy metal ions on the development of rancidity in blended cod muscle.

FIG. 2. Effect of adding metal ions on the development of rancidity in blended haddock muscle.

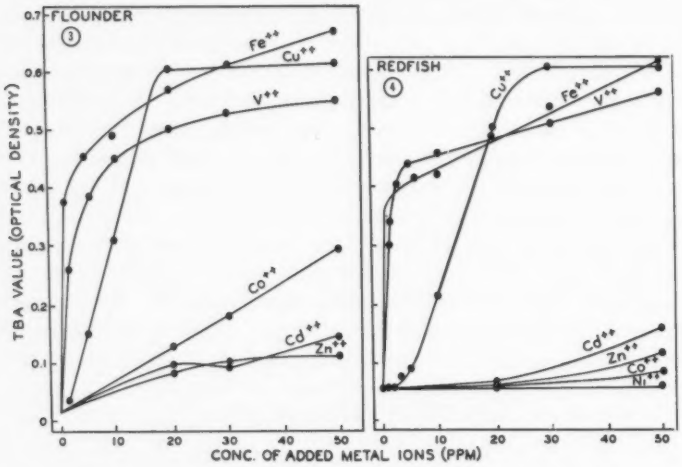

FIG. 3. Effect of adding heavy metal ions on the development of rancidity in blended American plaice muscle.

FIG. 4. Effect of adding metal ions on the development of rancidity in blended redfish muscle.

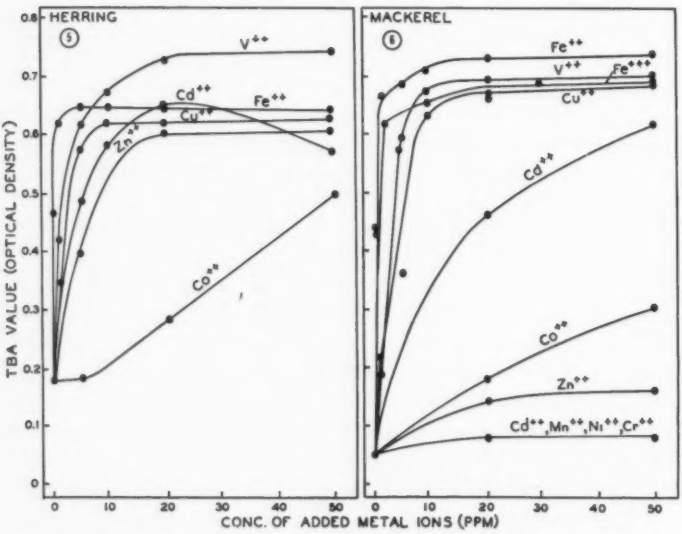

FIG. 5. Effect of adding heavy metal ions on the development of rancidity in blended herring muscle.

FIG. 6. Effect of adding heavy metal ions on the development of rancidity in blended mackerel muscle.

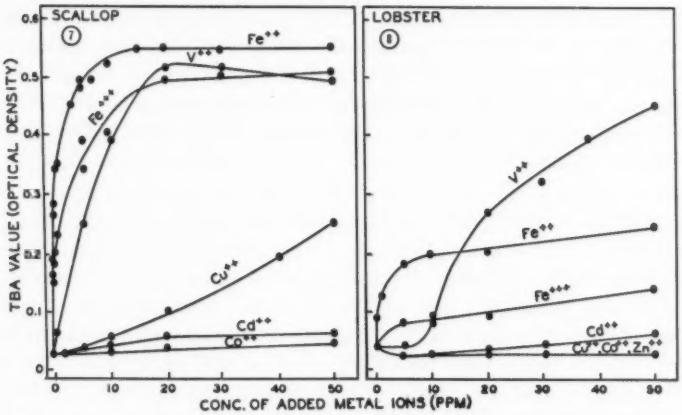

FIG. 7. Effect of adding metal ions on the development of rancidity in blended scallop muscle.

FIG. 8. Effect of adding metal ions on the development of rancidity in blended lobster tail muscle.

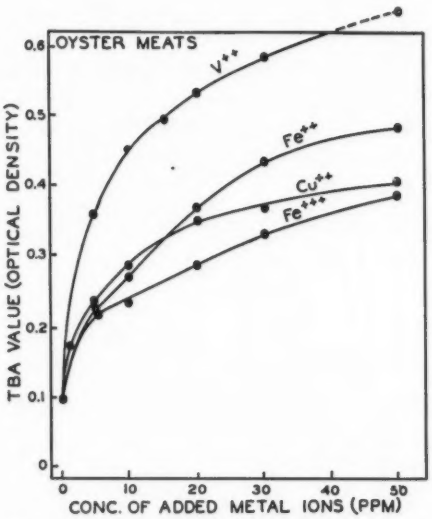

FIG. 9. Effect of adding heavy metal ions on the development of rancidity in blended oyster meats.

Special mention should be made of the comparative activity of Fe^{++} and Fe^{+++}. Without exception, Fe^{++} was more effective than Fe^{+++} in producing rancidity in muscle. But the relative activity of these iron ions varied from one species to another. In scallops (Fig. 7) and in the fatty fish (Fig. 6) the

activity of the ferric ion approached that of the ferrous ion; in the lean fish (Fig. 1) the ferric ion was comparatively much less effective. The maximum TBA values for the Fe^{+++} with fatty fish and scallops were higher than the maximum for the lean fish, suggesting that some component of the latter may have removed part of the ferric ions from the solution before they could react with the lipids.

Sensitivity of muscle from different species to rancidity produced by specific metals.— There were a few important exceptions to the general statements in the preceding section. Muscle from some species showed extreme sensitivity, and from others extreme inertness, towards the development of rancidity induced by certain specific metals. This can be most easily seen by a direct comparison of curves showing the effect of one metal on muscle from each of the different species that was tested. The most striking example is with Cu^{++} (Fig. 10). The herring and mackerel were quite sensitive to Cu^{++}: from 1 to 5 ppm produced strongly rancid odours and high TBA values. Cod and haddock were less susceptible, requiring from 10 to 20 ppm Cu^{++}, and occasionally 20–30 ppm, to bring about the same degree of rancidity. Scallop muscle was even less sensitive; and lobster muscle appeared to be strongly protected

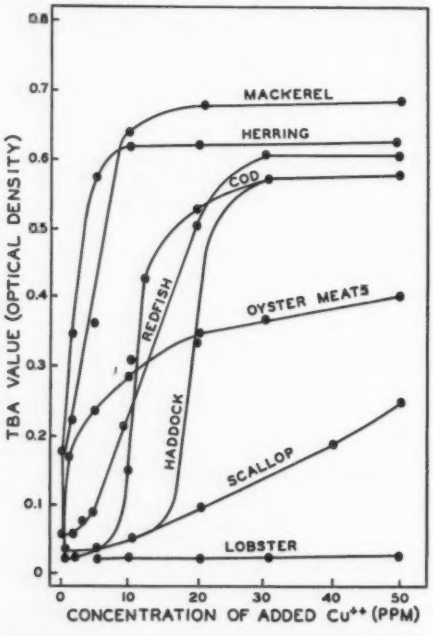

FIG. 10. Effect of concentrations of Cu^{++}, ranging between 0 and 50 ppm, on the development of rancidity in blended muscle prepared from eight different species of fish and shellfish.

against the action of Cu^{++}. This ability of Cu^{++} to induce rancidity in muscle from the different species cannot be correlated with their lipid content. The cod muscle had a lipid content of 0.7–0.9%, the lobster 0.9–1.1%, and the scallops 1.5–2.0%. Further tests were carried out with lobster muscle using higher levels of Cu^{++} (Fig. 11). Even 100 ppm caused little change in TBA values, and 100–200 ppm Cu^{++} caused only small increases in TBA value and slight off-odours, but none that were strongly rancid. Tests made on crab and shrimp muscle gave results similar to those with the lobster, suggesting that the crustacea as a group are protected against Cu^{++}-induced rancidity. In contrast to Cu^{++}, the effect of Cd^{++} appeared to be more closely related to the lipid content of the muscle from the different species (Fig. 12). The Cd^{++} produced most rancidity in the herring and mackerel, less in redfish and flounder, and had very little effect on the lipids in the muscle from the low-fat scallop, lobster, cod, and haddock.

Another interesting observation was the relative effectiveness of Fe^{++} and Cu^{++} to produce rancidity in scallop muscle (Fig. 7 and 13). Of all the species tested, scallop muscle was the most sensitive to Fe^{++} and Fe^{+++}; and, except for lobster and other crustaceae, the least sensitive to Cu^{++}. As little as a few parts per 100 million brought about significant increases in TBA values in scallop muscle (Fig. 14).

Induction periods for rancidity resulting from added Fe^{++} and Cu^{++}.— Figures 1 and 2 show that the relation between ion concentration and the development of rancidity in cod and haddock is quite similar for Fe^{++} and V^{++}, being logarithmic curves. In contrast to this, the results for Cu^{++} produce a sigmoid

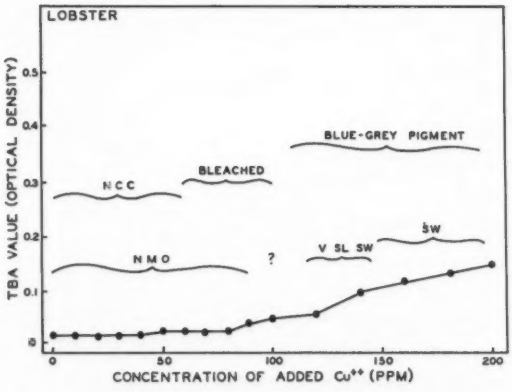

FIG. 11. Effect of various concentrations of Cu^{++}, between 0 and 200 ppm, on the development of rancidity and the bleaching of the muscle pigments in blended lobster tail muscle held for 24 hr at 0 C. NMO = not much odour; V Sl = very slight; Sw = seaweedy; NCC = no colour change.

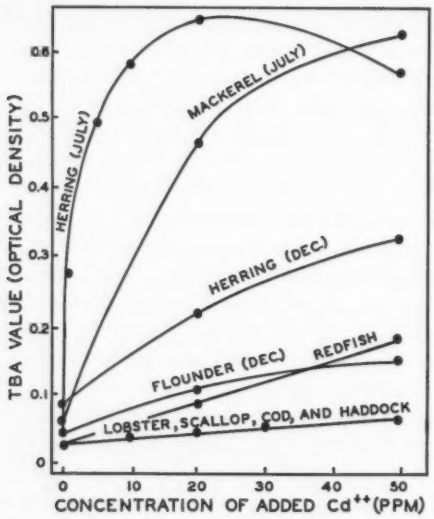

FIG. 12. Effect of various concentrations of Cd^{++} on the development of rancidity in blended muscle prepared from eight different species of fish and shellfish.

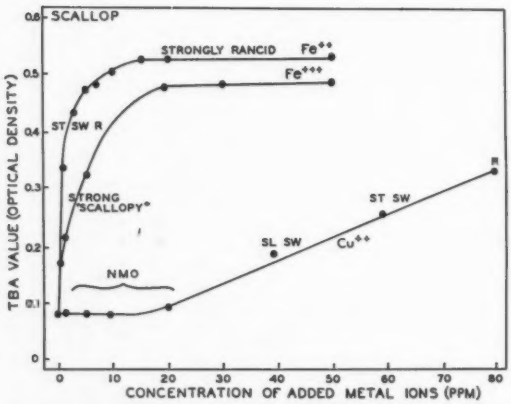

FIG. 13. Effect of the concentration of Fe^{++}, Fe^{+++}, and Cu^{++} on the development of rancidity in blended scallop muscle at 0 C for 24 hr. NMO = not much odour; Sl Sw = slight seaweedy; R = rancid; St Sw R = strong seaweedy rancid.

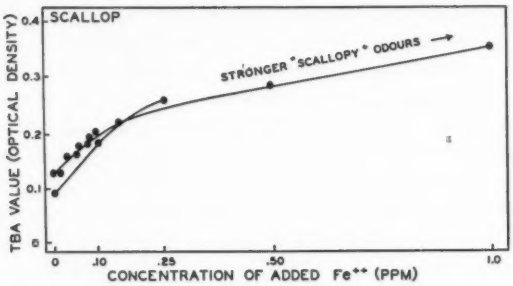

FIG. 14. Effect of very small concentrations of Fe^{++} on the TBA values of blended scallop muscle held for 24 hr at 0 C.

curve. Similar characteristic curves were obtained when TBA values were plotted against holding time, using only one concentration of metal ions (Fig. 15). It can be seen, therefore, that one difference in the action of these metals on the development of rancidity in the lean fish muscle is related to the induction period. It is extremely short for Fe^{++} and V^{++} and relatively long for Cu^{++}. In confirmation of this, initial rancid odours were observed in the blended cod or haddock muscle within 5 min after adding Fe^{++} or V^{++} (20 ppm), whereas similar odours resulting from the addition of Cu^{++} generally did not appear until 18–24 hr had elapsed. However, given sufficient time, the same concentration of Fe^{++} and Cu^{++} (20 ppm) ultimately produced similar high TBA values and equally rancid odours.

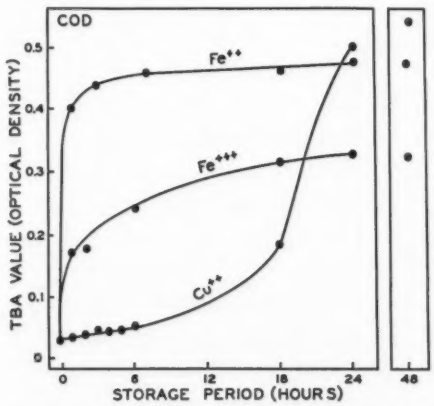

FIG. 15. Effect of time in storage at 0 C on the development of rancidity in blended cod muscle to which 20 ppm Cu^{++}, Fe^{++}, and Fe^{+++} had been added.

Effect of mixing muscle from different species.— The insensitivity of lobster muscle to Cu^{++}, the striking difference in the sensitivity of scallop muscle to Fe^{++} and Cu^{++}, and the long induction periods for Cu^{++} in cod and haddock suggested that the muscle from certain species may have natural mechanisms that protect their lipids against specific metals, and especially against Cu^{++}. It could be, as suggested by Lea (1952), that these tissues are able to bind the metal in such a way that it is no longer available as an oxidation catalyst. The lobster muscle, for example, may contain certain proteins, free amino acids, or other compounds that are able to combine with the Cu^{++} and render it inert as far as lipid oxidation is concerned. This raised the question as to whether the addition of lobster muscle to cod muscle would protect the cod lipids against Cu^{++}-induced rancidity; and if so, were the protective principles in the soluble, or in the insoluble components of the lobster muscle? Several tests that were made showed that the lobster did afford the cod muscle protection against Cu^{++}. An example is shown in Fig. 16. Four sets of muscle mixture containing various proportions of cod and lobster muscle were prepared. To each of these sets 30, 50, 100, and 200 ppm Cu^{++} were added. As the curves show, when the copper solutions were added to the cod muscle in the absence of the lobster muscle, high TBA values resulted and these all had corresponding strongly rancid odours. When 10–15% lobster was incorporated with the cod, the rancidity induced by 30 ppm Cu^{++} was almost completely retarded. As the concentration of Cu^{++} was increased, it required increasingly higher

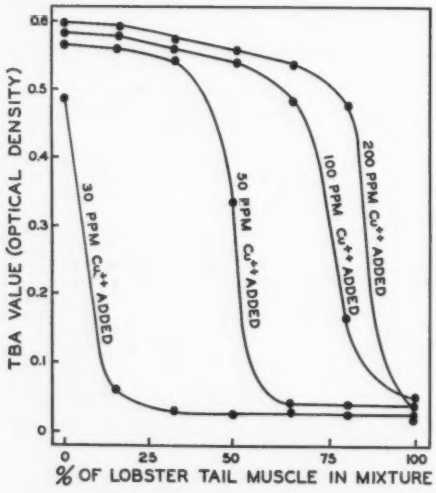

FIG. 16. The effect of mixing various proportions of cod and lobster muscle on the development of Cu^{++}-induced rancidity in these tissues. Separate experiments were carried out in which 30, 50, 100, and 200 ppm Cu^{++} were added.

proportions of lobster muscle to hold the rancidity in check. It can also be noted that in this particular experiment, with lobster muscle by itself, even 200 ppm Cu^{++} brought about very little change in the TBA value.

In another experiment, lobster muscle was blended with an equal weight of water and the liquid portion separated by centrifuging at 12,000 rpm for 30 min. The solids were twice resuspended and recentrifuged to remove most of the remaining solubles. Then an experiment, somewhat similar to that described above, was carried out, except that the soluble and insoluble fractions from the lobster muscle were added separately to two similar lots of cod muscle. Both the solids and the liquids from the lobster protected the cod lipids against added Cu^{++}. In this particular test the soluble portion afforded a little more protection than a corresponding amount of solids, but an accurate quantitative relationship remains to be worked out.

It was also observed that the order of mixing the cod and lobster muscle with the copper solution influenced the extent of rancidity that developed, suggesting competition between the two muscle tissues for the Cu^{++} (Fig. 17).

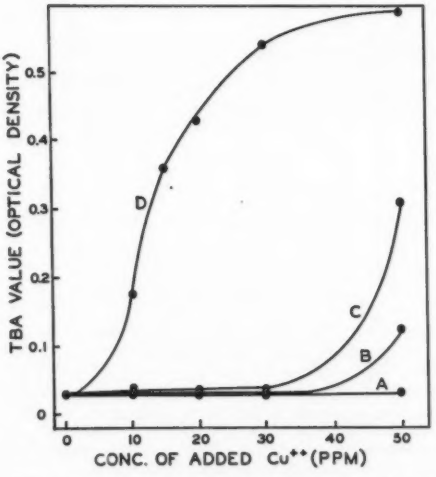

FIG. 17. Effect of the order of adding cod and lobster tail muscle and Cu^{++} on the development of rancidity. The cod and lobster muscle were added in equal amounts and series were run to which 0, 15, 20, 30, and 50 ppm Cu^{++} were added. A = lobster and copper solution mixed and cod added 10 min later; B = lobster, cod, and copper solution all mixed at the same time; C = cod and copper solution mixed and lobster added 10 min later; D = cod and copper solution mixed and lobster was omitted. When lobster and copper solution were mixed and the cod omitted the values were the same as for A.

Effect of freezing and storage on the susceptibility of fish muscle to metal-induced rancidity.— In a previous communication (Castell et al., 1966a) it was shown that during frozen storage cod fillets gradually decreased in their susceptibility to added Cu^{++} and that this change was directly related to the increase of free fatty acids (FFA) in the muscle. Since then, similar tests have been made with frozen muscle of other species in which FFA accumulates much more slowly (flounder and scallops) and the change in the susceptibility to the Cu^{++}-induced rancidity has been correspondingly retarded.

Frozen storage decreased the susceptibility of cod muscle to V^{++} and Fe^{++} as well as to Cu^{++}. The extent of the change was greatest for Cu^{++}, less for Fe^{++}, and least for V^{++} (Fig. 18).

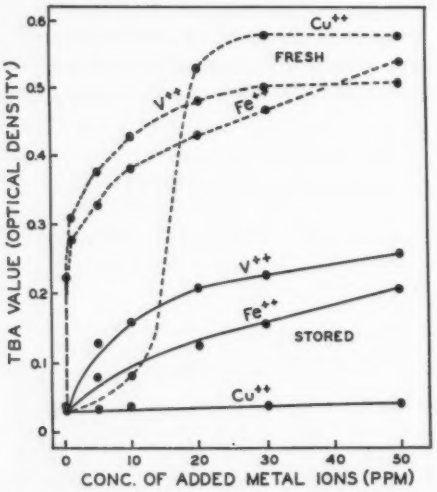

FIG. 18. Effect of prolonged frozen storage on the susceptibility of cod muscle to oxidative rancidity induced by adding from 0 to 50 ppm V^{++}, Fe^{++}, or Cu^{++}. The dotted lines are values obtained from fresh cod muscle. The continuous lines were obtained by making tests on cod muscle (wrapped fillets) after it had been in storage for 274 days at −12 C.

Effect of increasing the proportion of water in the blended muscle on its susceptibility to metal-induced rancidity.— One would expect that as the proportion of muscle in the blend is decreased, the proportion of lipid would also be decreased and the products of lipid oxidation would therefore be less. Experiments have shown that up to a certain point, rancidity increased as the proportion of muscle in the blend was decreased (Table I). There was still sufficient lipid in the blend to produce intense rancidity even when the muscle had been diluted by ten times its weight of water. It would appear

TABLE I. Effect of decreasing the proportion of fish muscle in the fish–water blends, on the development of metal-induced rancidity (using 20 ppm Cu^{++} and Fe^{++} and holding for 24 hr at 0 C).

Proportion	With added Cu++		With added Fe++	
F. muscle :water	TBA value	Odour	TBA value	Odour
1 :0.75	.145	NMO[a]	.190	Sl SFO
1 :1	.155	"	.190	" "
1 :1.5	.170	"	.230	" "
1 :2	.280	Sl Sw[a]	.220	Salt fish
1 :3	.240	Sl Sw	.265	Salt fish
1 :4	.320	Sw	.310	FSO[a] + Sw
1 :5	.400	Sw	.320	" "
1 :6	.500	St Sw	.310	" "
1 :10	.550	V St Sw R[a]	.410	SFO + St Sw

[a]NMO = not much odour; Sl Sw = slight seaweedy; V St Sw R = very strong seaweedy rancid; SFO = salt fish odour.

that dilution of the muscle also reduced some other component of the tissue that reacts with the metal ions to render them inactive as oxidation catalysts. It is the concentration of this other material, and not the concentration of the lipid that is the critical factor.

Effect of tocopherol on metal-induced rancidity.— α-Tocopherol is one of the principal natural antioxidants in fish lipids (Tsuchiya, 1961) and the concentration varies considerably from one species to another (Braekkan et al., 1963; Ackman and Cormier, 1967). Some experiments were carried out to determine whether added tocopherol would retard metal-induced rancidity in cod muscle blends and whether it was equally effective on rancidities induced by added Fe^{++}, V^{++}, and Cu^{++} (Fig. 19). The tocopherol was introduced into the blend in alcohol to help overcome the solubility problem. Although the concentrations in the blend may not be directly related to the concentrations in situ in the muscle, these results do show that this antioxidant is most effective against rancidity induced by Cu^{++} and is considerably less effective against Fe^{++} and V^{++}. These results and those from other similar experiments suggest that the concentration of tocopherol in tissues in different species may be an important factor in their susceptibility to metal-induced rancidities.

Odours accompanying metal-induced rancidities.— The identification of off-odours, as well as methods for determining and recording their intensity, are fraught with difficulties. It would be much easier to confine observations to objective measurements, but flavours and odours that accompany lipid oxidation are of very real importance to both the consumers and the producers of fishery products. An attempt will be made, therefore, to summarize some

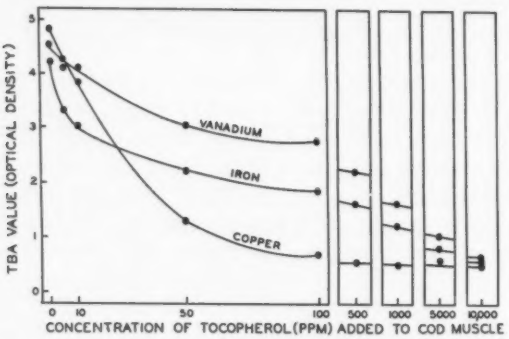

FIG. 19. The comparative effect of added α-tocopherol on rancidities induced by adding 20 ppm Cu^{++}, Fe^{++}, and V^{++}, as indicated by odours and TBA values after 24 hr at 0 C.

of the more important observations that have been found to accompany the development of various metal-induced rancidities in these fishery products.

(1) The development of rancidity in muscle tissue is always accompanied by a succession of off-odours which usually terminate in those we normally characterize as being "rancid" (i.e. suggesting old oily fish, strong cod liver oil, or oxidized linseed oil). Prior to this stage there are other off odours that are less likely to be associated with lipid oxidation. These include "salt-fish," "metallic," "seaweedy," and occasionally odours suggesting hydrogen sulphide. As oxidation progresses these different stages of odour development merge one into another. The following, for example, are the odours recorded for Cu^{++}-induced rancidity in cod muscle: "fresh fish odour" > "slightly off" > slight salt fish odour > seaweedy > seaweedy-rancid > rancid > strongly rancid (and occasionally) > "linseed oil" or "old paint odour."

(2) Although there is a similarity to the odours that develop, after the addition of V^{++}, Fe^{++}, and Cu^{++} to the muscle from any one species of fish, they are not identical. This is particularly true of the earlier, "pre-rancid" stages, where one odour usually predominates with each metal. This stage for Cu^{++}, in lean fish, is characterized by a "seaweedy" odour (which suggests the odours associated with the seaweeds on the shore at low tide); for V^{++} it is a "reedy" odour that is more easily recognized than described; with Fe^{++} it is "fishy" odours, ranging from that of salt fish to those suggesting trimethylamine. As the successive odours develop in muscle to which Fe^{++} has been added, this "fishiness" persists. Frequently our records show compound terms such as "fishy-seaweedy" and "fishy-rancid" where Fe^{++} has been added.

(3) The species from which the muscle was taken also influenced the type of odours that developed. Those from lean fish (cod, haddock, and winter-caught flounder) were quite similar and were characterized by having very pronounced "pre-rancid" odours. The fatty fish developed into the oily-

rancid stage much more rapidly. Rancidity in shucked oysters was accompanied by odours, difficult to identify and unlike those of any other product. Some muscles, such as the scallops, had quite pronounced odours when fresh. In this case the early stage of rancidity was accompanied by an intensification of the initial odour. Typical records for scallops undergoing rancidity (in the absence of bacterial activity) were as follows: fresh scallop odour > strong scallop odour > scallop odour plus cooked or spoiling vegetables > oily rancid.

DISCUSSION

Oxidative rancidity, as it develops in fish muscle, is more complex than rancidity developing in free fats or extracted oils. The reason for this is obvious. In muscle tissue, oxidation takes place under a more complex set of conditions. And when muscle samples are taken from different species of fish and other marine animals the complexity is compounded.

At this stage it would be difficult to account for all the variations that have been encountered in the metal-induced rancidities. The type, concentration, and distribution of the lipids in the muscle are undoubtedly important factors. The fish most susceptible to rancidity were the mackerel and herring. They had the highest lipid content, the most "free" fat, and the highest proportion of triglycerides. In contrast to these, the cod and haddock had the lowest lipid content, most of which is distributed in discrete portions of the muscle cell in the form of phospholipids and lipoproteins. Compared with the fatty species, these lean fish were less susceptible to rancidity. The "semi-fatty" flounder and redfish came between these two other groups.

However, the concentration or characteristics of the lipids were not sufficient to account for all the differences that were observed in muscle blends. The extreme insensitivity of the lobster muscle to metal-induced rancidities in general and to Cu^{++} in particular, needs some other explanation. And the lipid content of the scallop muscle cannot account for its widely different susceptibilities to Cu^{++} and Fe^{++}.

In addition to the lipids, consideration must be given to non-lipid components of the muscle which are capable of affecting or being affected by the reactions that take place as the lipids oxidize. These include compounds that accelerate lipid oxidation as well as those that retard it. The metals, of course, belong to this group. So also do many other compounds that can react with the metals and prevent their catalytic affect on the lipid oxidation. It was previously shown that the addition of cysteine, aspartic acid, tyrosine, tryptophane, and certain other amino acids had a strong inhibiting effect on copper-induced rancidity in blended cod muscle (Castell et al., 1966a). Many natural and artificial antioxidants function because of their ability to bind heavy metal ions. For a more complete explanation of the differences in the susceptibility of different muscles to metal-induced rancidity, one must include some of the non-lipid components.

In general, the relative effectiveness of the heavy metal ions in producing rancidity when added to the various fish muscles was of the following decreasing

order: $Fe^{++} > V^{++} > Cu^{++} > Fe^{+++} > Cd^{++} > Co^{++} > Zn^{++}$; in the concentrations used, Ni^{++}, Ce^{++}, Cr^{++}, and Mn^{++} had no effect. Of those tested, Fe^{++}, V^{++}, and Cu^{++} were by far the most active catalysts. However, having made this generalization it must be pointed out that there were important exceptions.The comparative effectiveness of the metallic ions was not the same for muscle taken from all the species that were tested.

In a similar general way it was found that the relative susceptibility of the muscle from the different species was of the following decreasing order: fatty fish (herring and mackerel) > semi-fat fish (redfish and flounder) > scallops > lean fish (cod and haddock) > crustaceae (lobster, shrimp, and crab). But once again this generalization had important exceptions where certain specific metals were involved.

Cod and haddock.— From 1 to 5 ppm Fe^{++} or V^{++} and usually 10–20 ppm Cu^{++} generally produced strong rancidity. In these fish, the induction periods for Fe^{++} and V^{++}-induced rancidities were very short whereas that for Cu^{++} was very much longer. The induction period for Fe^{+++} was of the same order as the Fe^{++}, but the maximum degree of rancidity was very much less. None of the other ions tested produced off-odours or significantly increased the TBA values when added to the lean fish.

Flounders and redfish.— The flounders may be included with either the lean or the semi-fat fish, depending upon the time of year they were caught. Tests on the lipid content of these flounders showed that during the late fall, winter, and early spring the values ranged between 1 and 1.5% and during the summer they were between 1.8 and 2.6%. The data recorded in Fig. 3 were from fish caught in December, with a fat content of approximately 1.4%. They were similar to the cod and haddock in being quite sensitive to Fe^{++} and V^{++}. They differed from these lean fish in being slightly more sensitive to Fe^{+++}, Cd^{++}, Co^{++}, and Z^{++}. The redfish gave results somewhat similar to those from the flounders.

Herring and mackerel.— As might be expected, the fatty fish were generally the most susceptible to the metal-induced rancidities. Their relation to Cu^{++} was of particular interest, in that it required a much lower concentration to induce rancidity and it developed faster. As we shift from the lean fish, through the semi-fat fish, to the very fatty fish the shape of the TBA–Cu^{++} concentration curves change from logarithmic to sigmoid. The induction period for Cu^{++}-induced rancidity in the fatty fish is very much shorter than that for the lean fish. The fatty fish are also more sensitive to rancidity induced by added Cd^{++}, Co^{++}, and Zn^{++}. The difference between the effect of Fe^{++} and Fe^{+++} was greatly reduced in the fatty fish.

Scallops.— The scallop muscle was characterized by its strong susceptibility to rancidity induced by either Fe^{++} or Fe^{+++} and its relative inertness to Cu^{++}; otherwise it was similar to the lean fish.

Oysters.— The tests with oysters differed from others in this series in that the whole shucked oyster was used and not the muscle alone. With the exception of the scallops and the lobsters, oysters were less sensitive to Cu^{++} than any of the other muscle samples tested. They were also relatively insensitive to Fe^{++}. One of the most interesting observations was that the off-odours accompanying the metal-induced rancidities in the oysters were quite unlike those of the fish muscle samples and are quite unlikely to be recognized as being "rancid." It was also noted that removal of the "liquid" that drains away from shucked oyster increased their susceptibility to rancidity.

Lobster.— The muscle from lobster was the most insensitive of all those tested to metal-induced rancidities in general and to Cu^{++} in particular. This was also observed with shrimp and several species of crab, indicating that it may be a characteristic of the crustaceae as a group. In place of hemoglobin, in the blood of these animals, there is a copper-protein pigment, hemocyanin. It has been suggested that this may be associated with their insensitivity to copper. However, it would not explain their insensitivity to other metals; nor does the presence of iron pigments in other animals make them any less sensitive to iron-induced rancidity. Ackman and Cormier (1967) found that the α-tocopherol content of the lipids in lobster claw and tail meat was very much greater than that of the lipids of the muscle of cod, flounder, mackerel, dogfish, and sablefish. The results of several experiments in this work with blended fish muscle have shown that added α-tocopherol was much more effective in suppressing Cu^{++}-induced rancidity than that caused by added Fe^{++} or V^{++}. We are inclined to believe, however, that many factors are involved in the development of rancidity in these various fish muscles.

It is worth pointing out again, especially in relation to lobster muscle, that the absence of rancidity, as indicated by TBA values and odours, does not mean freedom from other types of deterioration. Cu^{++}, Fe^{++}, V^{++}, and Co^{++} in concentrations that did not induce rancidity, did bleach the pigments; and the addition of Fe^{++} (20–100 ppm) frequently resulted in the development of odours suggesting cooked, spoiling vegetables and occasionally odours that were quite putrid. In every case these off-odours disappeared after 24 hr at 0 C and were not caused by microbial activity.

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Age and Growth of Round Whitefish (*Prosopium cylindraceum*) from Ungava[1]

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ABSTRACT

Growth in length and weight of round whitefish was similar in two localities in Ungava, northern Quebec. The pattern of growth, however, differed from those reported previously for round whitefish from the Great Lakes. Round whitefish from Ungava are slow growing and approach their maximum size at a constant growth rate, whereas those from the Great Lakes approach their upper size limit rapidly within the first 3 years and thereafter growth is slow. The maturing age was greater for both Ungava males and females (4+ and 3+ years respectively for the earliest specimens) than for round whitefish from the Great Lakes. Though round whitefish are abundant in the Ungava region, they are of no commercial interest because of their slow growth and small average size.

RÉSUMÉ

La croissance en longueur et en poids de *Prosopium cylindraceum* était semblable en deux localités de l'Ungava nord québécois. L'allure de croissance varie cependant comparativement à ceux précédement recontrés pour *P. cylindraceum* dans les Grand Lacs. Celui-ci approche rapidement de sa limite maximum pendant ses trois premières années de vie, et ensuite grandit lentement, alors que *P. cylindraceum* de la région d'Ungava fait preuve d'une croissance peu rapide a et approche de sa grandeur maximum à une allure constante. L'âge de maturation, et des mâles et des femelles de la région d'Ungava, est plus élevé (de 4+ ans et de 3+ ans respectivement pour les spécimens les plus avancés) que celui de *P. cylindraceum* dans les Grands Lacs. Bien que *P. cylindraceum* soit abondant dans la région d'Ungava, il ne peut avoir que peu de valeur commerciale car sa croissance est lente.

INTRODUCTION

This study on age and growth was carried out to describe the condition of stocks of round whitefish, *Prosopium cylindraceum*, living on the fringe of the tree line in Ungava, northern Quebec. Results were based mainly on data from the Koksoak River which flows northwards into Ungava Bay. A few data were collected from the George River to learn whether or not there were local differences in growth rate. Findings by Bailey (1963) for Lake Superior and Mraz (1964) for Lake Michigan were used to compare age and growth of round whitefish living under the climatically milder conditions of the Great Lakes with those of Ungava.

Though widely distributed in North America, the round whitefish has been one of the least studied of the coregonids. The first extensive growth studies for this species were made by Bailey (1963) and Mraz (1964) and until

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that time, only brief notes by Cooper and Fuller (1945), Kennedy (1949), and Rawson (1951) had appeared in the literature.

Dunbar and Hildebrand (1952) considered the round whitefish to be rare in the Ungava drainage, but Power (MS, 1958) reported this species as being abundant not only in the Koksoak River but also in the George River where it had never previously been listed. The apparent scarcity of the round whitefish may be a result of its small size which means that it is not often caught in the gillnets used by native fishermen. It may also be explained by the lack of distinction between the different whitefishes on the part of the natives.

MATERIALS AND METHODS

The samples used in the study consisted of 370 round whitefish from the Koksoak River obtained over a number of years from several locations and 61 round whitefish taken in 1956 at Helen Falls, George River (Table I). Totals shown in subsequent tables do not always agree with those in Table I because data on some specimens were incomplete.

The length of each specimen, from the tip of the snout to the end of the central ray of the caudal fin, was measured to the nearest millimetre. Both a beam balance and a spring balance were used for weight measurements in the field.

Sex and state of maturity were determined by examination of the gonads after dissection. Since the specimens were taken in the midsummer months when the gonads are unripe, it was often difficult to determine whether or not the fish would spawn the following winter. Data concerning state of maturity of the George River fish were not obtained.

The majority of fish were taken in nylon gillnets of 1.5-inch stretched mesh. The meshes were too large to take a representative sample of age-groups 1+ and 2+ and they selected the larger and faster-growing fish in age-group 3+. A rotenone sample was used to compensate for this inefficient sampling of the young age-groups.

TABLE I. Details of locality and capture of 431 round whitefish from Ungava.

Location	Year	Month	Gear	Number
George River System (total 61 fish)				
Helen Falls	1956	July–Aug.	Gillnets	61
Koksoak River System (total 370 fish)				
Whales Head and Maniton Gorge	1957	July–Aug.	Gillnets	16
Fort Chimo and Koksoak Island	1961	Sept.	Gillnets	16
			Rotenone	22
Koksoak River and Kaniapiskau River	1962	July	Gillnets	88
			Rotenone	63
Koksoak River	1964	Sept.	Rotenone	57
Koksoak River	1966	July–Aug.	Gillnets	108

The rotenone sample was also used to check the validity of the scale method to determine the age of fish. This was done by using the sample to construct a length-frequency histogram (Fig. 1), the peaks of which corresponded with the numbers of annuli on the scales.

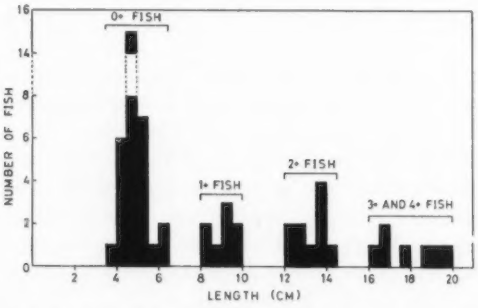

FIG. 1. Length-frequency distribution of a rotenone sample of 57 round whitefish taken in September, 1964; used to establish the validity of the scale method of age determination.

Scales from the left side of the fish anterior to the base of the dorsal fin and above the lateral line were read at the magnification ×30. Ages were read as the numbers of annuli on the scales and the presence of marginal growth was indicated by a plus sign. Scale radii and growth fields within annuli were measured along the longitudinal axis passing through the focus. These measurements were used to estimate annual growth of individual fish by the method of back-calculation for the Koksoak River sample. The body-scale relationship was found by fitting a straight line to a plot of average lengths and average scale radii. The equation for the regression line calculated by the method of least mean squares is

$$Y = -1.4513 + 0.4923X$$

where Y is the scale radius and X is the fork length in centimetres. This gives a positive intercept value of 2.95 cm or 1.16 inches on the X axis which is almost the same as that (2.79 cm) estimated by Bailey (1963) for round whitefish from Lake Superior. This intercept value of 2.95 cm was used as a correction factor when calculating past yearly lengths.

The length–weight relationship was expressed by the equation

$$W = {}_cL^n$$

where W is weight in grams; L is fork length in centimetres; and c and n are constants. Equations were determined for the Koksoak and George River

populations by fitting a straight line to the logarithm of weight values and the unchanged values of length using the method of least mean squares. The length–weight relationships are described by the equations:

$$\text{Koksoak River: } \log W = .06502L + 0.5106$$

$$\text{George River: } \log W = .06043L + 0.6173.$$

The weights were computed for the mean lengths of fish in each centimetre interval. The agreement of calculated and observed weights (Table V) was good except for intervals where there were few fish.

The annual growth rates of round whitefish from the Koksoak River (Table IV) were determined by applying the calculated annual lengths to the length–weight equation for the Koksoak River. It was not possible to calculate annual lengths and weights of round whitefish for the George River population because the sample was small with a limited size-range and an accurate body-scale relationship could not be determined.

GROWTH

There were slight differences in the average lengths of age-groups between the gillnet samples from the Koksoak and George Rivers and greater differences in age composition (Table II). Observed and calculated growth data (Tables II and III respectively) agree rather well but for conformity the calculated growth data were used for comparison with published Great Lakes data.

Growth in length of Koksoak River round whitefish is slow compared with that of round whitefish from the Great Lakes (Table IV). Those from Lake Michigan reach a length of 30 cm (approximately 1 ft) near the end of the third growing season. Those from Lake Superior atain the same length in their 5th year. In sharp contrast round whitefish from Ungava do not reach this size until their 9th year.

The mean annual increments in length (Table IV) reveal that the patterns of growth in the two areas are different especially concerning the earlier years. Those from the Great Lakes reach a size of 8.6–11.7 cm by the end of the first growing season, those from Ungava only 6.1 cm. After the rapid growth of the first 3 years the annual growth increments of round whitefish from the Great Lakes decline whereas the Ungava fish show no marked decrease in the annual growth increments in later years. The annual increases in length of the older Ungava fish equal and sometimes surpass those of the Great Lakes fish.

Growth in weight of the Koksoak River round whitefish appears to be almost the same as that of fish from the George River (Table V) but the differences between the calculated weights of the populations from Ungava and those from the Great Lakes are striking (Table IV). Weights attained by age-group 8 of round whitefish from Ungava are approximately 227 g (8 oz).

TABLE II. Age composition and observed length–age relationships of 228 round whitefish in gillnets from the Koksoak and of 60 from the George River.

Fork length (*cm*)	Age											
	1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	11+	12+
Koksoak River												
15.0–15.9		2										
16.0–16.9			4									
17.0–17.9			12	2								
18.0–18.9			6	9								
19.0–19.9			1	15	3							
20.0–20.9				20	6							
21.0–21.9				14	14	1						
22.0–22.9				2	14	2						
23.0–23.9				2	8	5						
24.0–24.9					5	3						
25.0–25.9					1	5	4	1				
26.0–26.9						2	3	1				
27.0–27.9							4	1				
28.0–28.9							2	6	2			
29.0–29.9								4	2	1		
30.0–30.9								2	6	2		
31.0–31.9								2	3	2		
32.0–32.9								3	2	5	1	
33.0–33.9										2		
34.0–34.9										1		
35.0–35.9											1	
36.0–36.9											1	2
37.0–37.9											1	3
No. of fish:	0	2	23	64	51	18	13	20	15	13	4	5
Percentage:		0.9	10.1	28.1	22.4	7.9	5.7	8.8	6.6	5.7	1.7	2.1
Avg length:		15.2	17.5	20.1	22.1	24.1	26.6	29.4	30.5	32.0	35.2	37.0
George River												
No. of fish:	0	2	2	6	13	16	12	6	0	1	2	
Percentage:	0	3.3	3.3	10.0	21.7	26.7	20.0	10.0	0	1.7	3.3	
Avg length:		11.7	15.0	20.3	22.3	24.6	26.1	28.3		34.0	34.7	

Fish from Lake Superior reach 227 g in 5 years whereas those from Lake Michigan reach the same in 3 years.

AGE AND LENGTH AT MATURITY AND SEX RATIO

The shortest mature males in the Koksoak River sample were 20.0–21.0 cm long and all males had apparently reached maturity by the time they were 25.0–26.0 cm long. The shortest mature females were 19.0–20.0 cm long and at 23.0–24.0 cm all had apparently reached maturity. The youngest mature male fish belonged to age-group 4+ and the youngest mature female fish to age-group 3+. All males were mature at age-group 7+ and all females at age-group 6+.

TABLE III. Calculated average total length at end of each year of life of 265 round whitefish (185 from gillnet and 80 from rotenone samples) from Koksoak River, Ungava.

Age-group	No. of fish	Length (*cm*) at end of year											
		1	2	3	4	5	6	7	8	9	10	11	12
1	21	6.1											
2	13	6.2	10.2										
3	27	6.1	10.2	14.8									
4	66	6.2	9.7	13.8	17.4								
5	51	6.2	9.6	13.3	16.9	20.1							
6	17	6.0	9.2	12.6	15.9	18.9	21.9						
7	13	6.0	9.6	13.5	16.8	19.7	22.3	25.0					
8	20	6.2	10.0	13.4	16.8	20.0	23.1	24.6	27.9				
9	15	6.2	9.6	13.2	16.4	19.2	22.0	24.6	26.7	28.8			
10	13	6.1	9.9	13.5	16.3	19.3	21.9	24.1	26.4	28.6	30.4		
11	4	6.2	9.6	13.6	16.9	20.0	23.3	26.1	28.5	30.4	32.1	33.8	
12	5	5.9	9.7	13.2	16.9	19.9	22.6	25.7	28.1	30.1	32.0	34.1	35.9
Mean:		6.1	9.7	13.6	16.9	19.7	22.4	25.0	27.3	29.1	31.1	34.0	35.9
Mean increment:		6.1	3.6	3.9	3.4	3.1	2.9	2.6	2.3	2.1	1.8	1.9	1.8
Sum of mean increments:		6.1	9.7	13.6	17.0	20.1	23.0	25.6	27.9	30.0	31.8	33.7	35.5

In the Great Lakes male round whitefish mature at an earlier age and smaller size than females. Bailey (1963) reported that the youngest mature males from Lake Superior belonged to age-group 2+ with lengths between 17.7 and 18.8 cm. The youngest mature females belonged to age-group 2+ and their lengths lay between 21.6 and 22.6 cm. Round whitefish from Lake Michigan (Mraz, 1964) showed differences in both size and age at maturity. The youngest mature males belonged to age-group 2+ and were between 30.5 and 31.5 cm long whereas the youngest mature females belonged to age-group 3+ with lengths between 33.0 and 34.0 cm.

The apparent conclusion that Ungava females may mature before males and at a smaller size may be an error due to the difficulty in estimating state of maturity. However, it is certain that round whitefish from the Koksoak River reach maturity later than but at a similar size to those from the Great Lakes.

Sex ratios were different in the Koksoak River and Lake Superior (Bailey, 1963) samples. The males outnumbered the females to age-group 5 in the Apostle Islands sample and then decreased to constitute only 20% of age-group 9. In the Koksoak River sample the females tended to outnumber the males except in age-groups 1 and 8, the percentages in the various age-groups that were males being as follows:

Age-group:	1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	11+	12+	Totals
No. of specimens	8	13	25	66	49	17	12	17	14	11	4	4	241
Percentage males	50	38	40	45	47	29	17	53	29	45	25	20	41

TABLE IV. Calculated lengths and weights at end of each year of life of round whitefish from Ungava, Lake Michigan (Mraz, 1964) and Lake Superior (Bailey, 1963).

Year of life	Fork length and annual increment (*cm*)[a]				Weight (*g*)			
			Lake Superior				Lake Superior	
	Ungava: Koksoak River	Lake Michigan	Apostle Islands	Isle Royale	Ungava: Koksoak River	Lake Michigan	Apostle Islands	Isle Royale
1	6.1(6.1)	11.7(11.7)	11.7(11.7)	8.6(8.6)	8.1	11.4	11.4	2.9
2	9.7(3.6)	22.9(11.2)	18.3(6.6)	15.2(6.6)	13.8	74.4	42.9	22.9
3	13.6(3.9)	31.2(8.3)	23.1(4.8)	21.1(4.9)	24.8	211.6	88.7	65.8
4	17.0(3.4)	36.3(5.1)	27.4(4.3)	26.2(5.1)	41.3	343.2	154.4	134.4
5	20.1(3.1)	39.6(3.3)	30.7(3.3)	30.7(4.5)	65.7	460.5	225.9	225.9
6	23.0(2.9)	42.7(3.1)	33.3(2.6)	34.0(3.3)	101.3	586.3	288.9	311.7
7	25.6(2.6)	45.5(2.8)	35.6(2.3)	36.6(2.6)	149.5	721.6	366.4	394.7
8	27.9(2.3)	48.0(2.5)	37.6(2.0)	38.9(2.3)	210.7	866.6	429.0	477.6
9	30.0(2.1)		39.4(1.8)	40.6(1.7)	288.8		497.6	552.0
10	31.8(1.8)			42.7(2.1)	378.1			646.4
11	33.7(1.9)			43.9(1.2)	503.0			707.3
12	35.5(1.8)			45.7(1.8)	658.6			786.5

[a]Annual increment in parentheses.

TABLE V. Length–weight relationships of 253 round whitefish from the Koksoak and 46 from the George River. The lengths and observed weights are averages for 1-cm intervals of length. The calculated weights were determined from the equations given in the text.

Koksoak River				George River			
No. of fish	Fork length (*cm*)	Weight (g)		No. of fish	Fork length (*cm*)	Weight (g)	
		Observed	Calculated			Observed	Calculated
3	8.3	5.0	11.2				
5	9.3	6.5	13.0				
				1	11.0	12.0	19.1
7	12.5	18.2	21.0	1	12.5	16.0	23.6
5	13.7	23.0	25.2				
1	14.0	20.0	26.3	1	14.5	28.0	31.8
1	15.9	34.3	33.8	1	15.5	32.0	35.8
7	16.5	35.5	39.6				
15	17.5	50.0	46.0				
16	18.5	58.4	51.7				
20	19.4	63.7	59.1	1	19.5	74.0	62.4
26	20.3	75.8	67.7	2	20.2	57.1	68.8
29	21.4	89.8	79.7	3	21.3	90.6	80.1
18	22.4	102.0	92.6	5	22.0	96.7	88.3
15	23.3	119.4	106.0	4	23.2	120.5	104.3
8	24.2	130.6	121.3	6	24.2	130.1	120.0
11	25.4	156.0	145.1	5	25.4	162.1	142.0
6	26.1	174.3	160.9	7	26.3	165.1	160.6
5	27.2	209.8	190.0	2	27.0	162.4	177.0
10	28.6	241.8	234.7	1	28.5	200.0	218.0
7	29.5	262.0	268.4	3	29.0	223.0	234.0
10	30.4	299.2	306.6				
7	31.5	306.5	361.1				
10	32.3	359.9	398.7				
2	33.1	458.5	459.8				
1	34.0	324.0	526.7	2	34.0	357.1	469.0
1	35.2	465.0	630.4	1	35.5	371.4	575.0
3	36.3	568.3	742.3				
4	37.4	531.7	876.2				

DISCUSSION

Although the growth patterns of round whitefish from Ungava and from the Great Lakes show that they are highly influenced by environment, the species appears to have the potential of reaching an ultimate size of 40–50 cm in both cases. The slow-growing populations, such as those from Ungava, approach the ultimate size at a steady, although slow, growth rate but few fish live to reach this maximum size. In a more moderate environment like that of the Great Lakes, the upper limit is approached rapidly at first and thereafter growth is slow.

A number of authors have tried to make general rules concerning the relationship between state of maturity, size, and age of fish. Several authors (Van Oosten, 1938; Van Oosten and Hile, 1947; Kennedy, 1953) are of the opinion that fish from slow-growing populations mature earlier and have a shorter life span than those from faster growing populations. Alm (1959), on the other hand, states that maturity appears at a lower age and at a smaller size in a population with a good growth rate and that, in a population with a poor growth rate, maturity is reached later and at a larger size. Neither of these "rules" seems to hold in this case. Admittedly the age at maturity is higher in the small forms from Ungava but they are reaching maturity at almost the same size as those from Lake Superior.

The smaller size at a given age of round whitefish from Ungava, their delayed sexual maturity, and their long life span could be due to the low temperatures at which they live. Gunter (1950) has proved that there exists a correlation between water temperature and the size at a given age of several species of fish along the east coast of North America. However, it is more likely that it is not one but a complex number of factors which influence the growth patterns in different populations.

The gradual drop in the percentage of males in later age-groups of fish populations is well known and as yet unexplained. Many investigations have shown that the sex ratio in young fish is unity or favours the males as in the Apostle Islands sample where the males outnumbered the females up to age-group 4. In older fish, however, the females invariably predominate. Many fisheries biologists have associated early maturity with a faster metabolic rate and hence a higher mortality..Alm (1959) disagrees with this hypothesis because experiments in ponds and lakes have given no evidence of a greater mortality of males. He assumes that the prolongation of life of the males is due to the absence of fishing and predators in the experimental ponds and lakes and not to any physiological reason. In order to explain the higher mortality of males in the wild he suggests that because males are more active and remain for longer periods of time at the spawning ground they are more exposed to predation and this results in a reduction of their numbers in the older age-groups. This explanation may well apply to the Ungava round whitefish.

Bailey (1963) and Mraz (1964) investigated the possibility of increased exploitation of round whitefish in Lakes Superior and Michigan. They set the minimum acceptable market weight at 453 g (1 lb). Lake Michigan round whitefish reach this weight in 5 years and Lake Superior fish in 8 years, but it is over 10 years before the round whitefish of Ungava reach this weight. It is obvious from the information presented here that there is no possibility of commercial exploitation of the round whitefish in Ungava.

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Species Succession and Fishery Exploitation in the Great Lakes[1,2]

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ABSTRACT

The species composition of fish in the Great Lakes has undergone continual change since the earliest records. Some changes were caused by enrichment of the environment, but others primarily by an intensive and selective fishery for certain species. Major changes related to the fishery were less frequent before the late 1930's than in recent years and involved few species. Lake sturgeon (*Acipenser fulvescens*) were overexploited knowingly during the late 1800's because they interfered with fishing for preferred species; sturgeon were greatly reduced in all lakes by the early 1900's. Heavy exploitation accompanied sharp declines of lake herring (*Leucichthys artedi*) in Lake Erie during the 1920's and lake whitefish (*Coregonus clupeaformis*) in Lake Huron during the 1930's. A rapid succession of fish species in Lakes Huron, Michigan, and Superior that started about 1940 has been caused by selective predation by the sea lamprey (*Petromyzon marinus*) on native predatory species, and the resultant shifting emphasis of the fishery and species interaction as various species declined. Lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*), the deep-water predators, were depleted first; this favored their prey, the chubs (*Leucichthys* spp.). The seven species of chubs were influenced differently according to differences in size. Fishing emphasis and predation by sea lampreys were selective for the largest species of chubs as lake trout and burbot declined. A single slow-growing chub, the bloater, was favored and increased, but as the large chubs declined the bloater was exploited by a new trawl fishery. The growth rate and size of the bloater increased, making it more vulnerable to conventional gillnet fishery and lamprey predation. This situation in Lakes Michigan and Huron favored the small alewife (*Alosa pseudoharengus*) which had recently become established in the upper Great Lakes, and the alewife increased rapidly and dominated the fish stocks of the lakes. The successive collapses of various stocks after periods of stable production may give some indication of their sustainable yield. The sea lamprey is being brought under control in Lakes Superior, Michigan, and Huron; lake trout are being established; and chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), kokanee salmon (*O. nerka*), and the splake, a hybrid of lake trout and brook trout (*Salvelinus fontinalis*), are being introduced to reestablish a new species balance. Fish stocks are in a state of extreme instability in these lakes. Careful control of stocking programs and fisheries, and coordination of management among the various states of the United States and the province of Canada (Ontario) which manage the fish stocks, will be required to restore and maintain a useful fishery balance.

INTRODUCTION

It was not apparent until recent years that the intensive and selective fishery of the Great Lakes over the past century has been a significant factor in a major modification of fish stocks. A succession of fish species would be expected during the natural aging process of the Great Lakes, but recent progressive

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changes in species composition have been rapid and obviously accelerated by influences of man, both by enrichment of the environment with wastes and by despoilment of the most abundant or preferred species of fish.

The species composition of fish in the Great Lakes has undergone continual change since the earliest records (Smith and Snell, 1891) but never have changes been more rapid or more drastic than in recent years. Environmental changes in Lake Erie (Beeton, 1961) and the related changes in the composition of the fish population and other aquatic organisms (Smith, 1962) have received considerable publicity in recent years. Modifications of fish stocks in the upper Great Lakes (Superior, Michigan, and Huron), which are deeper and more oligothophic, have been equal to or greater than changes in Lake Erie, but appear not closely related to modification of the physical–chemical environment. Although the water quality of the upper Great Lakes has undergone some changes (Beeton, 1965), these have not been the primary cause of what appears, with few exceptions, to be an irreversible succession of the major fish species. Recent changes have led to a state of biological instability in the mid-1960's that is almost unparalleled in the history of fishery science.

EARLY CHANGES

Since its beginning over a century ago the fishery of the Great Lakes has always shown preference for species that were abundant and in high demand. Less than a dozen species have been major contributors to the catch and, despite an intensive selective fishery, major changes in these species were few and local until recent years. Those that were influenced greatly by the fishery before the late 1930's were the lake sturgeon (*Acipenser fulvescens*), lake herring (*Leucichthys artedi*[3]), and lake whitefish (*Coregonus clupeaformis*).

Lake Sturgeon

The lake sturgeon was the first species affected by intensive exploitation and the only one that was knowingly depleted by man. Although the commercial value of the sturgeon was not great, they were abundant in all of the Great Lakes before 1900, particularly near shore where fishing for other species was heavy. These large fish frequently damaged gear fished for more valuable species, and were fished heavily to remove them from the fishing grounds (Harkness and Dymond, 1961). The recorded catches of sturgeon in the late 1800's exceeded 1 million lb annually in each of Lakes Michigan, Huron, and Erie, and often reached several hundred thousand pounds in Lakes Ontario

[3]Some authors include *Leucichthys* (ciscoes) in the genus *Coregonus* (whitefishes). I prefer the separate genera for clarity, and because a recent acrylamide electrophoretic study of blood serum made by Richard Cheilevold at the University of Michigan showed that the serum proteins distinctly separate ciscoes and whitefishes of both North America and Europe; that ciscoes and whitefishes each produce identical electrophoretic patterns between the continents; and that differences between ciscoes and whitefishes of Europe are the same as the differences in North America. Thus it is evident that the two groups are genetically distinct; have had separate origins; have attained their circumpolar distribution independently; and deserve generic distinction.

and Superior (Baldwin and Saalfeld, 1962). The recorded catches represent an unknown fraction of the total poundage taken; many were killed and thrown back in the lake or left to rot on the beaches. As a consequence of their slow growth and late maturity the accumulated stocks of adult fish were soon reduced to insignificance and became so scarce by the 1920's that sturgeon fishing was prohibited throughout most U.S. waters of Lakes Superior, Michigan, and Huron. Restrictions have been lifted in the State of Michigan waters of these lakes in recent years to permit the fishermen to keep sturgeon which are caught incidental to other fishing. The total production, however, is only a few thousand pounds in any of the lakes.

Partial protection of the sturgeon did not seem to increase its abundance noticeably, nor has an incidental fishery seemed to cause a decline. The only noticeable recent decline has been in Lake Erie, where catches have reached new lows since the mid-1950's, which may reflect recent unfavorable changes in the environment (Carr, 1962; Carr and Hiltunen, 1965; Carr et al., 1965). The lake sturgeon undoubtedly will continue to live in the Great Lakes at low abundance until influenced adversely by the changing environment, or by a fishery for another species that raises the incidental catch above the limit of its biological yield.

Lake Herring

The collapse of the lake herring population in Lake Erie in the mid-1920's (Van Oosten, 1930) was the first precipitous change in the fish stocks of the Great Lakes. Historically the lake herring has been very abundant and the most productive species in the Great Lakes — frequently contributing one-third to one-half of the catch of various lakes. In Lake Erie, lake herring were particularly abundant and were larger than in the other lakes. Because of these factors and the proximity of markets in large eastern cities, the fishery was intense. Before the collapse of the population the recorded catches were usually greater than 20 million lb annually and ranged as high as 49 million lb. Actual catches were much greater, however, as large quantities were frequently discarded during periods when catches were heavy and the market was glutted (Van Oosten, 1930).

The cause of the collapse of lake herring stocks in Lake Erie was never settled because detailed biological, ecological, and fishing data were lacking, but there are two pertinent factors that can be considered in retrospect. Fishing was very intense in the years preceding the decline and was particularly intense in the fall of 1924 — the year before the decline — due to an unusual concentration of lake herring in the east end of the lake (Van Oosten, 1930). After initial collapse the lake herring produced only one strong year-class — that hatched in 1944 (Scott, 1951) — although substantial spawning stocks were present until the mid-1950's. If the fishing rate before the decline was near that required for full utilization of the biological potential of the stock, and if recruitment was lowered due to reduction of spawning stocks or to

environmental variability in the early 1920's, the catch would have exceeded the maximum sustainable yield. This relation must lead to a swift decline in the stock (Ricker, 1963).

Although intensive exploitation and possible interaction with environmental change may have caused the sharp drop in lake herring stocks in the 1920's, a subsequent decline since the mid-1940's to extreme rarity approaching extinction in the mid-1960's undoubtedly is a response to increasingly unfavorable physical–chemical or biological environmental conditions. Fish collected in 1957 showed average 1st-year growth of lake herring of only 2.7 inches (Scott and Smith, 1962) which is much below the calculated 1st-year growth for lake herring in Lake Superior (4.7 inches; Dryer and Beil, 1964), Green Bay, Michigan (5.2 inches; Smith, 1956), or Saginaw Bay, Lake Huron (6.0 inches; Van Oosten, 1929). The slowest 1st-year growth recorded for the lake herring of 11 other lakes in the Great Lakes region was 3.4 inches (Carlander, 1953). Fish of Lake Erie, however, have been noted for their rapid growth. Examination of scales from Lake Erie lake herring showed that the average 1st-year growth was 7.2 inches — the highest value recorded for this species — for fish collected in 1945. The decline to 2.7 inches in 1957 provides strong evidence that the recent reduction of the lake herring in Lake Erie is more likely a result of environmental stress than a result of the small catches that have continued, and which have been mostly incidental to fishing for other species.

Lake Whitefish

The second collapse of a local fishery during the early period involved the development of a new type of gear — the deep trap net — that was very efficient for capture of lake whitefish in Lake Huron (Van Oosten et al., 1946). These nets were introduced in Lake Huron in 1928 at a time when whitefish abundance was high and apparently increasing. Beginning in 1930 their use expanded rapidly throughout the U.S. waters of the lake (this gear was never permitted in Canada), and the net proved particularly effective in central and southern Lake Huron. Over 4.1 million lb of whitefish were taken from the lake in 1931. As the nets were moved successively into various sections of the lake, local stocks were depleted rapidly. Thus, although stocks in different locations declined sharply, the total production of the lake did not decrease abruptly. The catch had declined to 1.9 million lb in 1935 when restrictions were imposed that prohibited setting trap nets in water deeper than 80 ft, but continued to decline to less than 0.1 million lb in 1942. Predation by the sea lamprey (*Petromyzon marinus*) discussed later may have been responsible in part for this low. Except for progeny of a very successful 1943 year-class that resulted in catches of about 3 million lb in U.S. waters in 1947 and 1948, the catch of lake whitefish has been less than 0.2 million lb in most years after the 1942 low (Baldwin and Saalfeld, 1962). A high mortality of the 1943 year-class and the recent low have been attributed to sea lamprey depredations rather than depletion through overfishing (Hile and Buettner, 1959).

Other Species

Although unrelated to exploitation, collapse of another fish stock which took place in early 1940's should be mentioned briefly. The American smelt (*Osmerus mordax*) was endemic in Lake Ontario but was introduced into the Lake Michigan drainage in 1912 and underwent a population explosion in Lakes Michigan and Huron in the 1930's (Van Oosten, 1937). The smelt population suffered a severe mortality during the fall and winter of 1942–43 probably due to a bacterial or virus disease (Van Oosten, 1947). The commercial production in Lake Michigan, where a thriving fishery for smelt had been established, fell from 4.8 million lb in 1941 to 4500 lb in 1944. The population recovered, however, and contributed to a very productive fishery as it became established in all of the lakes above Lake Ontario. The fishery was particularly productive in Lake Michigan where the catch reached a maximum of 9.1 million lb in 1958, and in Lake Erie where a peak catch of 19.2 million lb was produced in 1962.

A selective fishery for the larger chubs or deepwater ciscoes (*Leucichthys* spp.) influenced the species composition of this closely related group of species during the late 1800's and early 1900's. The early stages of these changes could not be measured because the various species of chubs were taken in the same net and were so similar that they were not sorted by the fishermen or listed separately in the catch statistics. But early records indicate clearly that the two largest species (*L. nigripinnis* and *L. johannae*) did, in fact, decline (Hile and Buettner, 1955). This early decline of certain species of chubs is discussed later in relation to the extreme modification of the chub population in recent years.

RECENT CHANGES

A rapid sequence of catastrophic changes which started in the 1940's led to a major alteration of the fish stocks in the Great Lakes. Until the 1940's the fishery of the Great Lakes as a whole was stable and productive despite loss of the sturgeon and the collapse of a few stocks in certain lakes. All preferred species continued in abundance somewhere in the Great Lakes; although many showed varying degrees of cyclical fluctuation, the composition of the total catch showed no marked changes or trends. The total production had stabilized and fluctuated only slightly around 100 million lb after declining from highs which often exceeded 140 million lb before 1920.

The major events that started in the 1940's involved the upper three lakes (Huron, Michigan, and Superior). The resulting succession of species resembled similar changes that apparently had taken place earlier in Lake Ontario, but which had gone almost unnoticed because the fishery was small and little studied. Changes that were progressing in Lake Erie, and which have accelerated since the 1940's, are not discussed here because both selective exploitation and environmental change were involved and their interactions are not understood.

The most intensive studies in the upper three lakes were made in Lake Michigan which is used here to illustrate similar events in Lakes Huron and Superior. The primary differences among these lakes have been timing and degree — in general, changes in Lake Huron started earlier and appear to have progressed further than Lake Michigan, and in Lake Superior they started later and have not advanced as far. The ultimate outcome may differ among the three lakes — their limnological characteristics differ somewhat, all are undergoing rapid change, and varying types and degrees of management are being imposed when each lake is at a different stage of change.

The Lake Michigan Fishery

The history of fishery exploitation in Lake Michigan was typical of the other Great Lakes. The highest annual production occurred near or before the turn of the century when the fishery had become well established. The catch exceeded 40 million lb in 4 of the 13 years for which statistics were available between 1897 and 1909 (Baldwin and Saalfeld, 1962), and averaged 35 million lb (Table I). In subsequent periods the total catch showed no marked variations or trends (Fig. 1) and averaged about 25 million lb until 1966 when the catch increased sharply to over 42 million lb due primarily to a greater catch of alewives. Nine species have been major contributors to the catch; they have constituted 95.6–99.8% of the catch in periods for which records of all species are complete (Table I).

At the turn of the century seven major species were represented in the catch (Table I). The lake trout (*Salvelinus namaycush*) and lake herring were the largest contributors, and the carp (*Cyprinus carpio*), which was introduced into the lake in the late 1800's, constituted less than 1% of the catch. Despite increased abundance of carp and the subsequent introduction and establishment of the smelt, the relative contribution of the native species to the catch showed no marked changes or trends until the 1945–49 period when the lake trout catch declined sharply. Subsequent species changes took place in swift succession (Fig. 1). In 1966, the catch was dominated by the alewife (*Alosa pseudoharengus*) which invaded the lake in the late 1940's (first recorded in 1949; Miller, 1957); exotic species constituted nearly 77% of the catch; and the portion of the catch composed of lake trout, lake herring, suckers (*Catostomus* spp. and *Moxostoma* spp.), and whitefish was only 4.4% as compared with more than 82% in the 1898–1909 period.

Several factors contributed to this extreme change. Much is known about these factors and their interactions although the exact mechanisms that brought about the change are incompletely understood. Unquestionably, predation of the sea lamprey initiated the events that led to the extreme decline of the lake trout in the upper Great Lakes. The resultant pressures of a shifting fishery and a population explosion of the alewife were factors of major significance. How these factors operated is illustrated by examining the changes in the deepwater stocks — lake trout and chubs — which occupy the area most strongly affected by sea lamprey predation, and the changes in the fishery.

TABLE I. Catch of major species and total commercial catch (thousands of pounds) per year in Lake Michigan for various periods.

Period	Native species						New species			Total major species			
	Lake trout	Lake herring	Suckers	Whitefish	Chubs	Yellow perch	Carp[a]	Smelt[b]	Alewife[c]	Pounds	Percentage of total catch	Other species	Total catch
1898–1909	7,225	16,977	1,810	2,715	2,061	3,866	261	–	–	34,915	–	– [d]	35,149
1910–1919	6,763	8,642	4,035	1,670	3,525	2,411	247	–	–	27,293	–	– [d]	27,059
1920–1929	7,001	4,015	2,117	2,111	2,902	1,537	679	–	–	20,362	–	– [d]	20,477
1930–1934	5,342	4,985	2,258	3,730	4,387	1,199	769	425	–	23,075	98.0	465	23,540
1935–1939	5,038	4,621	2,280	1,201	5,295	2,406	1,605	1,462	–	23,908	98.7	312	24,220
1940–1944	6,579	1,830	2,125	1,351	1,971	2,729	1,702	2,913	–	21,200	97.8	474	21,674
1945–1949	2,675	6,044	1,901	3,756	5,435	1,446	1,319	765	–	23,324	95.6	1,066	24,407
1950–1954	14	8,000	882	1,436	10,482	1,963	1,170	4,384	–	28,334	97.1	848	29,182
1955–1959	<1	3,639	652	105	9,947	3,055	1,785	6,983	568	26,735	97.8	592	27,327
1960	<1	233	767	124	12,695	3,285	1,416	3,267	2,370	24,158	99.4	153	24,311
1961	<1	177	494	412	12,133	4,959	1,842	2,152	3,199	25,370	99.3	189	25,559
1962	<1	116	263	278	11,115	4,050	1,206	1,546	4,742	23,317	99.3	158	23,475
1963	<1	41	299	298	7,460	4,872	1,277	1,203	5,396	20,795	98.9	226	21,021
1964	<1	34	215	788	5,172	5,835	1,320	969	11,744	26,080	99.5	121	26,201
1965	<1	47	168	995	7,441	1,297	2,016	927	14,007	26,900	99.7	94	26,994
1966	<1	49	402	1,422	7,228	654	2,713	1,110	29,002	42,580	99.8	97	42,677

[a]Introduced in the late 1800's.
[b]Introduced into Crystal Lake tributary to Lake Michigan in 1912.
[c]Invaded the upper Great Lakes via the Welland Canal and was first reported in Lake Michigan in 1949.
[d]Data incomplete.

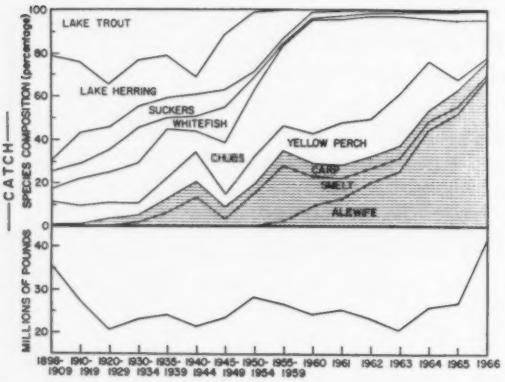

FIG. 1. Contribution of major species (percentage) and total commercial catch (pounds) of Lake Michigan for various periods 1898–1966. Shaded portion indicates exotic species.

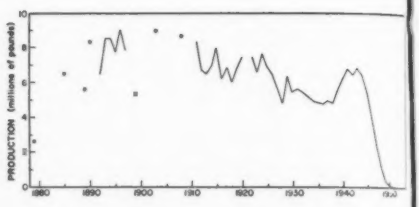

FIG. 2. Commercial production of lake trout in Lake Michigan for years of records 1879–1955

DECLINE OF THE LAKE TROUT

The lake trout has provided the most stable fishery of the Great Lakes. It was particularly productive in Lakes Superior, Huron, and Michigan, where catches usually exceeded 4, 5, and 6 million lb, respectively, and had not fluctuated widely before the declines in recent years. Biological information about the early stocks is not available. It is possible, however, as Kennedy (1954) has observed for lake trout stocks in Great Slave Lake, that recruitment was not strongly cyclic and that annual fluctuations of year-class strength were not great.

Lake trout production has been highest in Lake Michigan but the history of the catch was similar for all three lakes even though timing differed somewhat (Hile, 1949; Hile et al., 1951a, b). Catches in Lake Michigan increased in 1879–90 during the development of the fishery on the newly exploited stocks (Fig. 2). Production was highest during 1890–1911 and was characterized by periods of relative stability, in 1912–26 and 1927–39 at slightly lower levels (Hile et al., 1951a). The catch increased briefly in 1940–44, after which it underwent the collapse attributed to sea lamprey predation, and neared extinction by the mid-1950's due to a complete failure of natural reproduction after 1948 (Eschmeyer, 1957).

The collapse of the lake trout populations in the upper Great Lakes stimulated a massive effort to control the sea lamprey (Moffett, 1958). The early research has produced information on sea lamprey abundance during its population explosion that helps to interpret the relation between sea lamprey predation and lake trout decline, and the subsequent upset of the chub stocks. Although the measures of sea lamprey abundance over the longest period for each lake are based on spawning adults entering only one or a few streams (Table II), their agreement with trends in counts available for shorter

TABLE II. Number of sea lampreys counted during spawning runs in various streams in Lakes Huron, Michigan, and Superior, and their abundance index expressed as percentage of the average for the period of counts in each lake.[a]

	Lake Huron[b]		Lake Michigan[c]			Lake Superior[c]	
Year	Ocqueoc River	Abundance	Hibbards Creek	Three[d] streams	Abundance	Five[e] streams	Abundance
1944	4,488	50	–	–	–	–	–
1945	6,144	68	–	–	–	–	–
1946	8,072[f]	90	125	–	3	–	–
1947	10,000	111	596	–	16	–	–
1948	13,000	145	989	–	27	–	–
1949	24,634	275	1,579	–	43	–	–
1950	18,822	210	5,431	–	148	–	–
1951	19,393	216	12,640	–	345	–	–
1952	9,437	105	3,302	–	90	–	–
1953	11,676	130	9,247	–	252	1,424	24
1954	10,183	113	7,279	–	199	2,614	45
1955	13,683	153	6,395	18,279	164	3,096	53
1956	10,923[f]	122	5,325	19,653	155	8,048	138
1957	8,163	91	6,525	18,175	165	12,983	223
1958	5,465[f]	61	2,563	10,669	80	6,649	114
1959	2,766	31	2,330	8,636	68	8,224	141
1960	7,560	84	–	6,651	52	12,897	221
1961	4,715	53	–	12,886	101	13,305	228
1962	5,205	58	–	8,089	63	2,743	47
1963	4,674	52	–	7,461	59	3,838	66
1964	2,677	30	–	4,593	36	2,250	39
1965	1,390	15	–	3,277	26	2,205	38
1966	3,273	36	–	1,168	9	1,359	23

[a]The abundance for Lake Michigan was obtained by adjusting the total catch of the three streams to equal the catch of Hibbards Creek for the period of overlap (1955–59), averaging the figures for each year during this period, and extending the series based on the 1959 average and the percentage change from year to year for counts of the three streams.

[b]Records supplied by Dr Vernon C. Applegate.

[c]From reports of the Great Lakes Fishery Committee, and meeting reports and annual reports of the Great Lakes Fishery Commission available at the library of the Ann Arbor Laboratory, U.S. Bureau of Commercial Fisheries.

[d]Bark, Cedar, and Sturgeon rivers.

[e]Betsy River, Furnace Creek, Miners River, Sucker River, and Two Hearted River.

[f]No counts made; estimates are means of preceding and following years.

periods for other streams at various times suggests that these measures reflect closely the trends of abundance for each of the lakes.

The abundance of sea lampreys was very low in all three of the upper Great Lakes at the time when lake trout stocks started to decline (Fig. 3). The lake trout were unquestionably the prime prey of the sea lamprey as lake trout were the only abundant species of large fish that inhabited the colder (subthermocline) regions of the lakes preferred by the sea lamprey. Although large burbot (*Lota lota*), which occupied this area were less abundant

and suffered the same fate as the lake trout, it can be safely assumed that the trout suffered the brunt of the earliest predation. This predation could not have been great since spawning runs into the larger streams reportedly amounted to less than a hundred individuals before and during the early stages of lake trout decline (Vernon C. Applegate, personal communication).

The decline of lake trout in Lake Michigan was particularly abrupt, however, and may reflect the possible combined effect of increased predation by sea lampreys that were able to move freely from Lake Huron into Lake Michigan, and by the greater exploitation of lake trout by the commercial fishery in the period just before the decline. The increased catch occurred during the period when lake trout stocks were declining in Lake Huron and may be related in part to greater demand and higher prices at the time. Although the greater production may have been due in part to improvement in stocks since it was not accompanied by increased fishing effort in State of Michigan waters (Hile et al., 1951a), the higher production resulted mostly from the increased catch in Illinois which rose from 259 to 972 thousand lb (275% increase) between the periods 1927–39 and 1940–44. The Illinois catch had never been large and the increased catch of 713 thousand lb must have resulted in part from greater fishing intensity because of proximity to the Chicago market, the strong demand during World War II, and the declining catch in Lake Huron. It is also unlikely that the abundance increased to this degree in Illinois waters alone as sharp fluctuations in abundance were not characteristic of the lake trout and lake trout move freely throughout the lake (Smith and Van Oosten, 1940). This increase alone was more than 13% above the 1927–39 average for Lake Michigan, and if only half of it were attributed to greater fishing effort, it could have initiated a weakening of the lake trout stocks.

If fishing had been near the optimum rate in earlier years, only a small increase in exploitation would have been necessary to start a decline in the stocks. In an example based on biological characteristics similar to those of lake trout, Ricker (1963) determined that a 14% increase in exploitation separated maximum yield and no yield (collapse of the stock), and that half this increase (7%) would initiate a sharp decline. Thus, an increase of only 370–740 thousand lb over the average of about 5.3 million lb for the earlier period of stability during 1927–39 (Hile et al., 1951a) may have been sufficient to initiate the decline of the lake trout stock, which then became weakened further by sea lamprey predation.

Interaction of the Deepwater Fisheries

Until the establishment of the alewife in recent years, lake trout and chubs were the only species that were abundant in the deepwater area of Lake Michigan and important in the fishery. Although the interactions between the lake trout and chub fisheries were complex (Hile and Buettner, 1955), the two fisheries were essentially alternate; they usually employed the same boats and fished in much the same areas, but differed in the use of large-mesh gillnets for lake trout and small mesh for chubs.

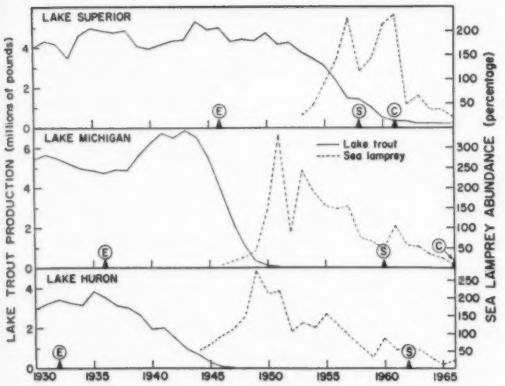

FIG. 3. Production of lake trout and abundance of the sea lamprey in Lakes Superior, Michigan, and Huron, and the time of first sea lamprey record or establishment (E), initiation of chemical control (S), and completion of the initial series of chemical treatment (C) in each Lake.

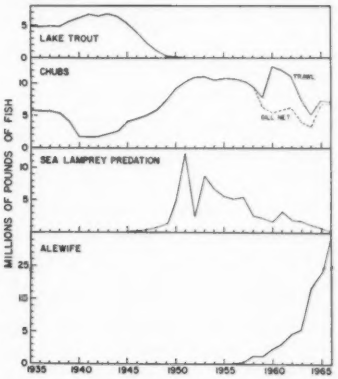

FIG. 4. Production of lake trout, chubs, and alewives, and the estimated pounds of fish destroyed by sea lampreys of the spawning population of Lake Michigan.

The chub fishery was depressed by reduced effort during the war years in 1940–44, but recovered as the lake trout declined; the change reflected a transfer of effort from lake trout to chubs (Hile and Buettner, 1955). The chub catch reached a record high of 7 million lb in 1949 and increased to a high period of production above 10 million lb during 1951–57 (Table III). It was during these same peak years of chub production that the sea lamprey attained its greatest abundance. Because the deep waters contained no other prey (lake trout and burbot had both disappeared) the chubs received the impact of sea lamprey predation (Moffett, 1957). The estimated pounds of fish killed by the number of sea lampreys that had survived to spawn exceeded 11 million lb in 1951 and was above 5 million lb until 1957 except for a low of 3 million lb in 1952. These estimates are conservative because they are based on counts of spawning-run sea lampreys and do not account for an unknown amount of predation by lampreys that did not survive to spawn.

The consequence of this extreme pressure of high commercial production and sea lamprey predation on the chub population was severe. Of the seven species of chubs that inhabited Lake Michigan the two largest (*Leucichthys johannae* and *L. nigripinnis*) had become extinct, and the four species of intermediate size (*L. alpenae*, *L. kiyi*, *L. reighardi*, and *L. zenithicus*) had been seriously depleted as shown in a survey during 1960–61 (Smith, 1964). The smallest and slowest growing chub, the bloater (*L. hoyi*), was favored during this period and became very abundant. It had been a primary food of the lake trout (Van Oosten and Deason, 1938) and most were too small to be taken profitably in commercial gillnets or serve as suitable prey for the sea lamprey. As the supply of larger chubs declined during the late 1950's, the commercial gillnet catch declined, and the abundance of sea lampreys

TABLE III. Production of lake trout, chubs, and alewives, and estimated pounds of fish destroyed by spawning run sea lampreys in Lake Michigan in 1935–66.

Year	Lake trout	Chubs			Alewife	Fish destroyed by sea lamprey[a]
		Gillnet	Trawl	Total		
		(*Thousands of pounds of fish*)				
1935	4,873	5,794	–	5,794	–	–
1936	4,763	5,674	–	5,674	–	–
1937	4,988	5,579	–	5,579	–	–
1938	4,906	5,404	–	5,404	–	–
1939	5,660	4,025	–	4,025	–	–
1940	6,266	1,648	–	1,648	–	–
1941	6,787	1,630	–	1,630	–	–
1942	6,484	1,755	–	1,755	–	–
1943	6,860	2,214	–	2,214	–	–
1944	6,498	2,607	–	2,607	–	–
1945	5,437	4,221	–	4,221	–	–
1946	3,974	4,525	–	4,525	–	116
1947	2,425	5,087	–	5,087	–	554
1948	1,197	5,929	–	5,929	–	919
1949	342	7,421	–	7,421	–	1,467
1950	54	9,291	–	9,291	–	5,046
1951	11	10,301	–	10,301	–	11,744
1952	4	11,098	–	11,098	–	3,068
1953	[b]	11,151	–	11,151	–	8,591
1954	[b]	10,568	–	10,568	–	6,762
1955	[b]	10,895	–	10,895	–	5,576
1956	0	10,913	–	10,913	[b]	5,275
1957	0	10,546	–	10,546	220	5,622
1958	0	9,583	–	9,583	1,357	2,711
1959	[b]	6,392	1,404	7,796	1,264	2,313
1960	[b]	5,530	7,129	12,695	2,370	1,782
1961	[b]	5,915	6,218	12,133	3,199	3,452
1962	[b]	6,346	4,769	11,115	4,742	2,167
1963	26	4,050	3,410	7,460	5,396	1,999
1964	[b]	3,381	1,791	5,172	11,744	1,230
1965	[b]	6,857	583	7,440	14,007	877
1966	[b]	6,990	238	7,228	29,002	313

[a]Based upon a proportional expansion of the abundance index (Table II) to reflect the sea lamprey count in 1958 when the maximum number of streams were blocked with counting barriers (Great Lakes Fishery Commission, 1958) which was doubled on the assumption that one-half of the spawning streams were blocked, and that each lamprey killed 37 lb of fish to reach maturity (Packer and Lennon, 1956).

[b]Less than 500 lb. Most lake trout caught since 1953 were hatchery-reared fish planted to measure survival and planting techniques.

and their predation decreased (Fig. 4), but the total chub catch increased to a new high of 12.7 million lb in 1960 (Table III). This increase resulted from the use of trawls which had not been employed in the Great Lakes fishery until they were introduced in the late 1950's to catch the small bloater which

had become very abundant by the mid-1950's (Moffett, 1957) and alewives which were starting to become abundant.

Most of the bloaters and the alewife were too small for the human food market, and trawling provided an efficient means of catching them in large quantity at low cost for animal food and fish meal. Markets for alewives were not well established, however, and the population had not increased sufficiently to support heavy production during all seasons. The trawl fishery was restricted to certain species and depths of fishing so that the only abundant species that could be taken by trawling were alewives and chubs. Thus, during the early stages of the trawl fishery bloaters composed the greater part of the catch (Table III) because they were very abundant, available during all seasons, brought a higher price than alewives on the animal-food market, and included some large chubs (about 7% of the catch) that brought a high price on the human-food market.

Even the gillnet fishery became dependent on bloaters when species of large chubs had become scarce during the late 1950's. The human-food market accepted smaller chubs at lower price, but the bloaters were also becoming larger (Tables IV and V). The average length of bloaters in both southern

TABLE IV. Length distribution (percentage) of bloaters taken in trawls in southern Lake Michigan at Grand Haven (1954, 1960) and Saugatuck (1962–66).

Length (*inches*)	Year						
	1954	1960	1962	1963	1964	1965	1966
2.5–2.9	0.1	–	–	–	–	–	–
3.0–3.4	0.1	–	–	–	–	–	–
3.5–3.9	0.5	–	–	–	–	–	–
4.0–4.4	1.2	T	T	–	T	–	–
4.5–4.9	1.3	T	0.2	–	T	–	0.1
5.0–5.4	1.2	0.1	T	T	–	T	0.3
5.5–5.9	10.8	0.2	0.1	0.1	T	–	0.1
6.0–6.4	22.5	0.8	–	0.1	T	0.1	0.1
6.5–6.9	23.8	3.0	0.6	0.2	0.1	0.1	0.1
7.0–7.4	12.7	20.1	9.2	3.3	3.6	2.0	0.9
7.5–7.9	10.3	32.3	35.0	26.4	25.6	17.7	10.1
8.0–8.4	6.4	19.9	28.4	34.6	32.6	28.4	23.8
8.5–8.9	4.9	11.5	13.8	17.2	19.2	22.9	26.3
9.0–9.4	2.4	7.3	6.8	10.0	10.4	15.9	20.3
9.5–9.9	1.2	3.4	3.8	4.9	5.7	7.5	10.7
10.0–10.4	0.5	1.1	1.6	2.3	1.9	3.7	5.1
10.5–10.9	0.1	0.2	0.4	0.7	0.6	1.3	1.5
11.0–11.4	–	0.1	0.1	0.2	0.2	0.3	0.5
11.5–11.9	–	–	–	–	0.1	0.1	0.1
12.0–12.4	–	–	–	–	–	T	–
Avg length:	6.86	7.98	8.15	8.36	8.38	8.59	8.78
No. of fish:	4,336	16,454	1,745	5,215	8,720	3,055	1,771
Percentage >9.0 inches:	4.2	12.1	12.7	18.1	18.9	28.8	38.2

TABLE V. Length distribution (percentage) of bloaters taken in trawls at Manistique in northern Lake Michigan in various years between 1955 and 1964.

Length (*inches*)	1955	1961	1964
5.0–5.4	0.8	–	–
5.5–5.9	0.3	–	–
6.0–6.4	1.3	0.1	–
6.5–6.9	1.8	–	–
7.0–7.4	6.7	2.8	0.4
7.5–7.9	13.2	11.5	1.9
8.0–8.4	26.9	28.7	9.9
8.5–8.9	24.0	24.3	20.8
9.0–9.4	12.4	17.0	29.5
9.5–9.9	7.7	10.2	18.7
10.0–10.4	2.8	3.7	11.2
10.5–10.9	1.5	1.3	5.1
11.0–11.4	0.3	0.2	1.7
11.5–11.9	0.3	0.2	0.6
12.0–12.4	–	–	0.2
Avg length:	8.46	8.68	9.30
No. of fish:	387	1635	475
Percentage >9.0 inches:	25.0	32.6	67.0

and northern Lake Michigan increased in every year for which data are available between 1954 and 1966. The mean length in southern Lake Michigan increased almost 2 inches during this period, and the percentage of bloaters large enough for the human-food market (usually >9 inches) increased from 4.2 to 38.2%. In northern Lake Michigan the length increased about 1 inch between 1955 and 1964, and the bloaters suited for human food rose from 25.0 to 67.0%. Since the average length of bloaters taken in 1960–61 increased from south to north (Table VI), it might be assumed that the length increased throughout the lake during the period of 1954–66. Preliminary studies show that this increase in average length reflects both an increase in average age and an increase in growth rate.

The combined influence of the new trawl fishery and the increase in size of bloaters which made them better suited for the gillnet fishery resulted in the largest chub catches of record in Lake Michigan in 1960–62. Sea lamprey predation of about 2 million lb or more during this period was also directed primarily at bloaters, many of which had increased to a size more vulnerable to lamprey predation.

The chub fishery declined sharply after this period of record catch (Fig. 4). Deaths from botulism in October 1963 of several persons who had eaten smoked chubs from the Great Lakes undoubtedly contributed to the low catches in 1963–64, but the catch through September 1963 had already declined

TABLE VI. Length distribution (percentage) of bloaters taken in trawls at various ports in Lake Michigan in 1960 and 1961.

Length (*inches*)	St. Joseph 1960	Milwaukee 1960	Grand Haven 1960	Frankfort 1961	Sturgeon Bay 1961	Charlevoix 1961	Manistique 1961
2.5–2.9	–	–	–	0.1	–	–	–
3.5–3.9	0.1	–	–	–	–	–	–
4.0–4.4	–	–	T	–	–	–	–
4.5–4.9	–	0.1	T	0.1	–	–	–
5.0–5.4	T	T	0.1	0.5	–	–	–
5.5–5.9	0.1	0.1	0.2	0.5	–	–	–
6.0–6.5	0.4	1.0	0.8	0.6	–	0.1	0.1
6.5–6.9	3.1	2.5	3.0	2.0	0.4	0.3	–
7.0–7.4	31.6	20.5	20.1	9.8	5.0	3.6	2.8
7.5–7.9	36.7	37.3	32.3	30.2	24.8	18.3	11.5
8.0–8.4	15.0	23.3	19.9	26.1	36.7	32.4	28.7
8.5–8.9	6.8	8.9	11.5	15.6	22.4	21.7	24.3
9.0–9.4	4.1	4.3	7.3	8.3	8.4	13.0	17.0
9.5–9.9	1.6	1.6	3.4	3.8	2.0	6.1	10.2
10.0–10.4	0.5	0.3	1.1	1.9	0.3	3.3	3.7
10.5–10.9	T	0.1	0.2	0.4	–	1.0	1.3
11.0–11.4	–	T	0.1	0.1	–	0.1	0.2
11.5–11.9	–	–	–	–	–	0.1	0.2
Avg length:	7.74	7.86	7.98	8.14	8.25	8.49	8.68
No. of fish:	3,570	4,228	16,454	2,077	1,039	1,226	1,635

27% from that of the comparable period in 1962. The increase between 1964 and 1965 probably reflects recovery of the market after the botulism scare and the sharp increase in availability of large bloaters (Tables IV and V).

INTERACTION AMONG DEEPWATER SPECIES

There is little question that the selective fishery in the Great Lakes has had a substantial influence on the interaction among various species (Hile, 1954). When ecological relationships are disturbed by selective fishing so that the reduction of some species gives a competitive advantage to others, a population imbalance is created and a rapid succession of species can follow. It is difficult to separate successions induced by environmental changes from those caused by exploitation because both factors are usually involved. The deepwater area of Lake Michigan has had no conspicuous environmental changes except in fish populations, however, and provides a good illustration of the interaction and succession of species influenced primarily by exploitation.

Unexploited stocks.— The Great Lakes are young; during less than 10,000 years in their present form they have developed a very simple deepwater fish fauna. The lake trout was the principal climax predator and it fed mostly on the young of six species of large chubs and a small slow-growing chub bloater (Fig. 5A). Adults of the larger chubs were mostly too large for prey

of lake trout and provided a breeding stock that added stability to the predator–prey balance.

The burbot was the only other predator in the deepwater region but it was less abundant than the lake trout. The lake trout and burbot also preyed heavily on the deepwater sculpin (*Myoxocephalus thompsonii*) and to a lesser extent on the ninespine stickleback (*Pungitius pungitius*) in the northern end of the lake (Van Oosten and Deason, 1938). Measures of abundance are not available for the burbot, sculpin, and stickleback and they cannot be included in this discussion, but it can be assumed that the burbot underwent changes similar to the lake trout. There has been no evidence of major change in the abundance of the deepwater sculpin (which occupies a very limited niche close to the bottom in the deeper areas thoughout the lake) or of the stickleback (largely restricted to the northern section of the lake).

Stable fishery, 1910–40.— The introduction of a lake trout fishery which underwent an initial increase and several subsequent levels of stability (Hile et al., 1951a) undoubtedly had the usual effect on a newly exploited stock

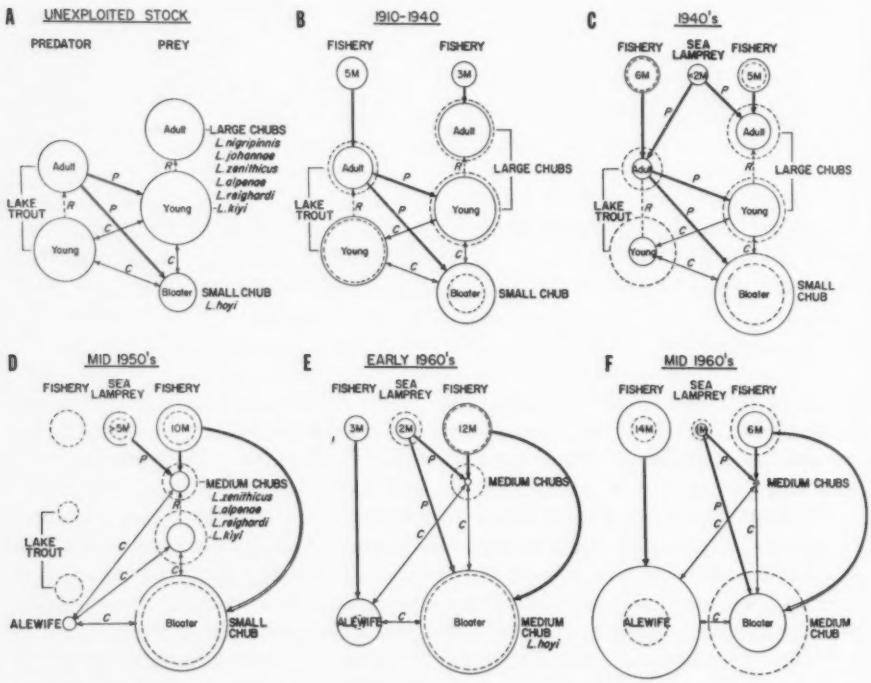

FIG. 5. Interrelations of major deepwater species of Lake Michigan before exploitation (A) and during the following periods: stable exploitation, 1910–40 (B); early influence of the sea lamprey, 1940's (C); maximum abundance of the sea lamprey, mid-1950's (D); maximum abundance of bloaters, early 1960's (E); maximum abundance of the alewife, mid-1960's (F). P = predation; C = food competition; and R = recruitment.

composed of many age-groups, and reduced the number of older and larger fish (Ricker, 1963). Data are lacking but scattered accounts indicate clearly that the largest lake trout were reduced and became legends by 1927–39 when the catch stabilized at about 5 million lb (Hile et al., 1951a). Since the lake trout experienced no major fluctuations it can be safely assumed that the stocks were not overexploited and that the population underwent the typical adjustment to lower average age, increased growth rate, and more rapid recruitment of young to adults which compensated for the exploitation of the fishery (Fig. 5B).

A modest fishery for chubs that fluctuated about a usual catch of some 3 million lb had a similar influence on the chub stocks. Two of the largest species of chubs, however, were selected strongly by the fishery and were reduced greatly by the early 1900's (Hile and Buettner, 1955). This reduction was apparently compensated by an increase in the representation of bloaters in the chub stocks as measured during a fishery survey in 1930–32 (Moffett, 1957). The increase in abundance of bloaters would have offset any reduction of young of the larger chubs that served as prey for lake trout, and thus contributed to the stability of the lake trout stock and the lake trout fishery.

Establishment of the sea lamprey, 1940's.— The sea lamprey became established in the deepwater population in small but significant numbers during the 1940's. Although the sea lamprey undoubtedly preyed on all large fish in the deepwater area (lake trout, burbot, and adults of large chub species), their effect on the lake trout population was swift and drastic. The estimated pounds of fish killed by the sea lamprey annually in the 1940's was small in comparison with later years and may not have exceeded 2 million lb at the end of the decade (Table III). Sea lamprey predation certainly must have been comparatively small when the lake trout catch started to decline about 1945 (Fig. 3). The increased exploitation by the fishery during the period immediately preceding the decline of lake trout, however, could have made the lake trout stock of Lake Michigan particularly sensitive to the predation by the sea lamprey.

As the lake trout declined, its predation on small chubs decreased and the sea lamprey predation turned from lake trout to the adults of large chubs. The fishery also transferred to large chubs as lake trout declined (Fig. 4). Thus the deepwater stocks of Lake Michigan, after supporting a stable fishery since its beginning, entered a period of instability favoring bloaters (Fig. 5C) which according to complaints of commercial fishermen had started to become a nuisance in their nets by 1950.

Sea lamprey maximum, mid-1950's.— Fishery surveys of Lake Michigan during 1950–55 showed that the two largest chubs had become nearly extinct; that the four remaining species of larger chubs were greatly reduced; that bloaters constituted over 75% of the chub population (Moffett, 1957); and that lake trout were near extinction (Eschmeyer, 1957). By the mid-1950's the sea lamprey population had reached maximum abundance; the estimated destruction exceeded 5 million lb of fish per year (Table III), primarily large

chubs. In this period of peak abundance, the sea lamprey was also observed more frequently in shallower areas of the lake where it preyed heavily on whitefish, suckers, and walleyes (*Stizostedion vitreum*), all of which suffered sharp reduction in stocks. The deepwater fishery which had only chubs to catch increased to over 10 million lb during 1951–57, the period of the sea lamprey maximum. The large chubs were preferred by the fishery but were insufficient to fill the demand so the larger bloaters started to enter the catch for the first time and were marketed as a "No. 2 chub" at a lower price.

The events of the mid-1950's produced great instability in the balance of deepwater species, created a strong pressure against the large chubs, and favored bloaters despite the first substantial exploitation of larger bloaters by the fishery (Fig. 5D). An almost unnoticed but significant event in the early 1950's was the establishment of the alewife which was first reported in 1949. No alewives were taken in extensive experimental trawling in southern Lake Michigan in 1954, but eight alewives were taken during a survey throughout the northern part of the lake in 1955. Over 45,000 and 38,000 alewives were taken in experimental trawls during similar surveys of southern and northern Lake Michigan in 1960 and 1961, respectively.

Bloater maximum, early 1960's.— Bloaters dominated the chub population even more strongly according to surveys in 1960–61, when they constituted 93–95% of the chubs taken in experimental gillnets (Smith, 1964). The abundance of bloaters had increased in the latter period at four of the seven locations used for 1954–55 and 1960–61 comparisons. The size of bloaters had increased during this period (Tables IV and V) and, since the largest individuals of the other species of chubs were gone, much of the ecological and fishery significance of size differences among species of chubs had been lost. Also exploitation of all sizes of chubs started with the initiation of trawling, as chubs constituted well over half of the catch taken by trawls in 1960–61 when the total chub catch had reached record highs above 12 million lb (Table III). The larger chubs of all species were still preferred, however, and were exploited heavily by the gillnet fishery. The sea lamprey, which had suffered a population decline as its prey declined, still destroyed more than 2 million lb of fish annually in the early 1960's.

Thus, the pressures had shifted to bloaters but continued heavy on the small populations of the four larger chub species that remained in the lake (Fig. 5E). The alewife was the only species present in deeper areas of the lake that could be favored by the selection against chubs. It had become well established throughout the lake by the early 1960's and supported an expanding fishery of about 3 million lb.

Alewife maximum, mid-1960's.— The competitive advantage of the alewife which resulted from heavy exploitation of chubs of all sizes undoubtedly speeded a population explosion of alewives that gained its greatest impetus in the early 1960's. The number of young-of-the-year alewives taken in comparable trawling during early November in southern Lake Michigan increased 56 times between 1962 and 1964, and the number of adults increased 5 times

between 1962 and 1966 (Table VII). The small change in abundance of adults, the increase in average age of the adult stock between 1965 and 1966, and the failure of the extremely large 1964 year-class to increase the adult stocks in 1966 indicate clearly that the alewife population was approaching saturation.

The great abundance of alewives and a continuing heavy exploitation of chubs had placed the chubs at a strong disadvantage by the mid-1960's (Fig. 5F). It is not certain if the fishery for alewives that reached 29 million lb in 1966 was large enough to influence the alewife stocks sufficiently to affect their competitive advantage.

Measures of the abundance of chubs have not been available since 1960–61 but the dominance of bloaters continued to increase, and they constituted 99.2% of all chubs taken in experimental trawls during 1964 (Wells, 1966). Evidence also exists of a biological change of uncertain causes that may reflect an ecological stress for bloaters. In addition to the marked increase in size of bloaters which reflects the combined effect of faster growth and an increase in average age in recent years, there has also been a continued increase in percentage of females in the population. The proportion of females in the population reached 97–99% during various months in 1966.

An increase in size has accompanied declines in populations of the lake herring, a close relative of the bloater. The size and growth rate of the lake herring of Lake Superior increased during early stages of a recent decline in numbers of fish in the stock in 1950–62 (Dryer and Beil, 1964), and the size has continued to increase as the abundance declined sharply after 1962. A well-documented study of the collapse of a lake herring population in Birch Lake, Michigan, showed that an increase in growth and size of fish in the population, and a strong dominance of females (84–93%) preceded a sharp decline in 1945–46 (Clady, 1967). Although data are lacking, the collapse of lake herring stocks of Lake Michigan in the late 1950's also was accompanied by observations of fishermen and biologists of a progressive increase in the size of fish taken in the fishery. The lake herring of Lake Michigan were presumed to have declined as a result of early establishment of alewives in shallow water (Miller, 1957). Such evidence implies that the recent biological changes of the bloater stocks of Lake Michigan may be indicators of a pending collapse of the population. There is no indication among coregonids that these changes have stopped or reversed until after the population has declined.

Establishment and Dominance of the Alewife

The alewife has been the only fish in the Great Lakes that has occupied all sections of a lake and its bays, estuaries, and tributaries in great numbers. It has become the most abundant and widely distributed species in Lake Michigan where it has displaced all major planktivorous species. Although studies are lacking, similar dominance of the lake and other species appears to have occurred in Lakes Ontario and Huron.

The alewife was first recorded in the Great Lakes in Lake Ontario in 1873 and has been abundant there since the late 1800's. Just how the alewife

TABLE VII. Abundance of adult and young-of-the-year alewives in Lake Michigan at Saugatuck, expressed as catch per 10-min trawl tow and as effective catch per unit of effort (CPE) for depths of 3–40 fath during early November in 1962–66.

	Young-of-the-year					Adult				
Depth	Nov. 8, 9, 1962	Nov. 7, 9, 1963	Nov. 4, 1964	Nov. 10, 1965	Nov. 15, 1966	Nov. 8, 9, 1962	Nov. 7, 9, 1963	Nov. 4, 1964	Nov. 10,[a] 1965	Nov. 15,[b] 1966
3	–	12	1714	2340	0	–	64	18	406	80
5	7	254	1478	2638	4	294	182	176	260	514
7	27	357	2599	2500	42	18	111	28	657	911
10	48	25	107	946	587	80	52	198	803	752
12	–	78	319	925	518	–	62	238	497	429
15	74	299	1591	22	320	12	107	308	507	534
17	30	204	1989	0	462	110	42	204	798	805
20	28	5	5863	0	2	26	74	396	507	529
25	4	3	0	0	2	57	206	106	227	400
30	0	0	0	0	1	76	60	34	232	219
35	0	0	1	0	0	182	14	52	156	84
40	0	0	0	0	0	1	12	56	143	141
Effective CPE[c]	31	137	1740	1562	215	86	82	151	432	450

[a]Percentage age composition: I, 2%; II, 73%; III, 25%.
[b]Percentage age composition: I, 1%; II, 59%; III, 38%; IV, 2%.
[c]Average catch for depths at which fish were taken.

got into Lake Ontario is uncertain. It long had free access to the lake via the St. Lawrence River from its native habitat along the Atlantic Coast. It seems that the alewife would have appeared in Lake Ontario much earlier if it had followed this route. It is more likely that alewives that became established in Lake Ontario were included in shipments of young American shad (*Alosa sapidissima*) that were planted in Lake Ontario in the early 1870's (Woods, 1960).

After its establishment in Lake Ontario, the movement of the alewife to the other Great Lakes was slow. Niagara Falls was a natural barrier to fish migration between Lakes Ontario and Erie before the completion of the Welland Canal in 1829. Contrary to common belief, this canal did not provide an easy route for fish to bypass Niagara Falls. Its many locks, which raise ships 327 ft between Lakes Ontario and Erie, impose partial barriers and the strong downstream currents created as the huge locks are emptied repeatedly, are major deterrents to upstream migration. The sea lamprey, a native of Lake Ontario, took nearly 100 years after the canal was opened to appear in Lake Erie in 1921 (Applegate, 1950), and the alewife did not appear in Lake Erie until 1931 — 58 years after it was first found in Lake Ontario. When the alewife reached Lake Erie it had free access to all of the other Great Lakes. It was first found in Lake Huron in 1933, in Lake Michigan in 1949, and in Lake Superior in 1954.

The alewife was common in Lake Erie by 1942, but it probably did not become extremely abundant there. The deep water where the alewives concentrate in winter to avoid unfavorably cold water is limited in Lake Erie and the lake has always had a large population of predators. Lake Huron also had a large population of lake trout and burbot that would have preyed on the alewife when it entered the lake in the 1930's, but these were virtually eliminated from the lake during the mid-1940's by sea lamprey predation. Subsequently the alewife became very abundant by the mid-1950's and increased irregularly until 1964 (Coble, 1967).

The alewife has spread throughout Lake Superior but has not yet become abundant and its future there is uncertain. The lake not only has a large population of predators, but is the coldest of the Great Lakes — and cold does not favor the alewife.

The sea lamprey had almost eliminated the large predators in Lake Michigan when the alewife was first recorded there in 1949. The alewife had strong competitive advantage over other planktivorous species because of its efficient filter-feeding behavior and its characteristic of forming dense schools that occur in all areas of the lake during different seasons.

The alewife increased rapidly in Lake Michigan during the 1950's; it was first noted in abundance in Green Bay in 1953, and was abundant throughout the lake in 1956. During recent years the adult population has more than doubled each year. Obviously this rate of increase could not continue as each new hatch of young encounters an increasingly greater population density and more competition for food and space. Scattered data on age composition

indicates that a decreasing percentage of young survived to become adults as the population increased. As this trend continues to a point where the number of adults is so great that it becomes very difficult for young to survive, the number of young reaching adulthood starts to decrease and the population reaches a peak. Evidence indicates that the population had reached this point during 1966–67.

Because the alewife occupies various sections of the lake in dense schools during different seasons, it can influence all other species of the lake. In mid-winter the adult alewives are densely concentrated on the bottom in the deepest areas (Wells, 1968), where they seek the warmest waters. This area was occupied by the kiyi (*L. kiyi*), which was abundant as recently as 1954–55 (Moffett, 1957), but had become greatly reduced by 1960–61 (Smith, 1964), and was nearly extinct by 1964 (Wells, 1966).

In late winter and early spring the alewives move toward shore in dense schools that pass through the region occupied by the bloater (Wells, 1968). This region is also occupied by the alewife in the fall as it returns to deep water. Although the bloaters increased greatly during the 1950's (Moffett, 1957; Smith, 1964), their abundance has been decreasing sharply in the 1960's as alewives increased.

During the late spring and summer the adult alewives are concentrated in the shallower areas (Wells, 1968), where they spawn near shore and in rivers and bays during June and July. The lake herring and the emerald shiner (*Notropis atherinoides*), which previously were extremely abundant in the shallow areas and bays of the Great Lakes, decreased sharply in various areas as alewives became established (Miller, 1957) and were very scarce or absent after the alewife reached extreme abundance in Lake Michigan (Wells, 1968).

The yellow perch (*Perca flavescens*) which occupies the inshore area became more abundant as the alewives increased in Lake Michigan. The increase in abundance and resultant stimulation of effort were reflected by an increasing catch of perch during the early 1960's that reached 5.8 million lb in 1964. The increase in yellow perch may have resulted from the greater supply of food that the alewives provided for larger perch. As alewives became very abundant they crowded the shore in dense schools during spring and summer and the yellow perch moved further from shore. The concentration of alewives near shore, and the displacement of yellow perch occurred during the spawning and hatching period of the yellow perch, and as the alewives became very abundant during the early 1960's recruitment of perch must have been affected as the abundance of adult perch dropped sharply. This was reflected by the sharp decline of the catch of yellow perch to 1.3 and 0.7 million lb in 1965 and 1966, respectively.

The American smelt which also occupied the inshore areas of the lakes declined sharply when alewife abundance increased. The smelt production in Lake Michigan declined from a peak of about 9.1 million lb in 1958 to 0.9–1.2 million lb since 1963. Because the American smelt is still abundant in Lake Ontario where the alewife has long been the dominant fish, it seems

unlikely that smelt will be influenced as strongly by the alewife as were the chubs, lake herring, and emerald shiners, all of which are very scarce in Lake Ontario.

During the fall and winter after hatching, the young alewives occupy the mid-depths of the lake where they remain until their third summer (age II) before moving to the bottom. The midwater area has also been the zone occupied by the young lake herring, chubs, and American smelt during their first 2 years. Undoubtedly the competition between the young of the alewives and the young of other species was severe as the alewives reached great abundance.

The occupation of various zones of the lake in different periods by dense congregations of alewives has changed greatly the utilization of the invertebrate productivity by fish. The chubs, lake herring, emerald shiners, yellow perch, and American smelt were abundant species that occupied various sections of the lake and were major consumers of invertebrates throughout the year. Under present conditions when the alewife is concentrated inshore in the summer, the deepest zone previously inhabited by the kiyi in great abundance (Hile and Deason, 1947) is essentially unoccupied by an abundant species and the invertebrate food is incompletely used. Similar situations occur during other seasons when sections of the lake vacated by the alewives as they move from zone to zone are left with greatly reduced populations of the species that once occupied each section in great abundance throughout the year. As abundant as the alewife is, and despite its apparent intensive use of the invertebrate food of each zone of the lake during different seasons, I believe it unlikely that the biomass represented by the alewife is equal to the total of all of the previously abundant species that it has displaced.

Reestablishment of Climax Predators

The unprecedented instability of fish stocks in Lake Michigan in the mid-1960's at a time when the alewife fishery is continuing to expand and climax predators are being reestablished certainly will result in continued rapid change. Although the alewife catch reached 29 million lb in 1966 and may reach 50 million lb in 1967, processing facilities that exist can handle approximately 100 million lb. Despite the heavy annual die-off of alewives each spring that was the greatest on record in 1967, alewives continued to be plentiful during the summer of 1967. It is uncertain if the fishery approached full exploitation of the alewife stocks at their peak. Early decrease in alewife stocks seems likely, however, due to over-population and a sharp increase in mortality between the end of the 1st year and entry into the adult stocks, as illustrated by the fewer adults that entered the stocks at age II from the very large 1964 year-class (266 in 1966) than from the much smaller 1963 year-class (315 in 1965; Table VII).

A vigorous program is underway to reestablish climax predators in Lake Michigan to create a predator–prey balance with the alewife. Sea lamprey control in Lake Michigan was initiated in 1960 and was effective in 1967.

Lake trout stocking by the states and federal government started in 1965, has reached nearly 2 million fingerlings annually, and will be continued until natural reproduction is well established. Michigan stocked 660,000 fingerling coho salmon (*Oncorhynchus kisutch*) in Lake Michigan in 1966, and 1,700,000 coho and 800,000 chinook salmon (*O. tshawytscha*) in 1967. The survival of coho salmon as indicated by spawning runs of adults from the 1966 plant and precocious males of the 1967 plant indicate unusual success. Michigan and Wisconsin are increasing their plants of steelhead trout (*Salmo gairdneri*) in Lake Michigan tributaries. The coho salmon are occupying the upper strata of the lake and are feeding heavily on the young alewives that live at mid-depths. This region is apparently where the steelhead trout and chinook salmon will feed most heavily as neither are taken on the bottom in deeper water.

The Province of Ontario has started to make large introductions of splake in Lake Huron and these fish will have free access to Lake Michigan if successful. The splake is a hybrid of lake trout and brook trout (*Salvelinus fontinalis*) that has been selected for several generations to develop a strain that will occupy deep water, grow fast, and mature early. Michigan and Ontario have also initiated experimental introductions of kokanee (*Oncorhynchus nerka*) in the Lake Michigan watershed and in Georgian Bay of Lake Huron. Although it is uncertain where the kokanee will live in the Great Lakes, they will probably compete with the alewife for food.

It seems unlikely that all of these species can be established with equal success as the productivity of Lake Michigan has not changed greatly over the pre-sea lamprey period. The closed ecosystem of a lake differs greatly from the native ocean habitat of the salmon, and competition among the various species of salmon and trout for the available food will undoubtedly become intense as their numbers increase. It is uncertain yet which predators will have a biological or competitive advantage over others, but it does appear that the predator–prey relation will be unstable as long as the alewife remains the single dominant prey species. In fact, it seems likely that the fishery productivity may be lowered from the pre-sea lamprey period because of the instability of the predator–prey balance, and apparent less-efficient use of the invertebrate food of the entire lake by the alewife than by the previous multiple-species complex.

Greater stability and full productivity of Lake Michigan, and other Great Lakes that have been similarly affected by recent events, can only be achieved by careful regulation of the kinds and numbers of predators, and the reestablishment of a multiple-species complex of prey species. Since the greatest stability occurs at low population density, management of the lakes to obtain the highest yield will require continuous surveillance and manipulation of all species to prevent onset of uncontrolled instability and recurrence of extreme imbalances in the future.

To manage the fishery resource for high yield, both recreational and commercial fisheries must be closely regulated and the amounts of various

species taken by each must be closely controlled with respect to the potential yield of each species and the expected interactions among competing predator or prey species that are not fished heavily or selectively. It may also be necessary to create or induce fisheries for species not involved directly in the predator–prey balance of the lakes, but which could be favored by short- or long-term changes of the environment.

An essential requirement for successful and continued restoration of a useful fishery balance is uniformity in management approach among the states and Canada which own and manage the fisheries in their respective sections of the various lakes. Management objectives for individual lakes or adjoining lakes must be compatible and regulations and management procedures closely integrated.

The potential of common attack on problems of the Great Lakes is illustrated by the highly successful interstate and international program to control the sea lamprey and rehabilitate the lake trout that has been, in fact, the first step toward the achievement of a new and more favorable ecological balance among fish stocks.

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Interaction of the Pathogen *Gaffkya homari* with Natural Defense Mechanisms of *Homarus americanus*[1]

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ABSTRACT

Infection of the lobster *Homarus americanus* with the bacterium *Gaffkya homari* invariably proved fatal. Very small numbers of this pathogen introduced into the hemolymph resulted in septicemia and death, i.e., a dose level as low as approximately five bacteria per lobster at a temperature of 15 C was sufficient to kill 90% of the lobsters in 17 days. The mean time to death was virtually constant, regardless of the dose, indicating a general lack of host defense against *G. homari*.

Investigation of the interaction of *G. homari* with the defense factors apparent in lobster hemolymph (bactericidal, agglutinin, and phagocytic activities) helped to account for its pathogenicity. *Gaffkya homari* was unique among the microorganisms tested in that (1) it was resistant to the agglutinin(s); (2) its growth was stimulated by the hemolymph serum rather than inhibited or unaffected; (3) although it was phagocytized, the bacterium could overcome this effect and multiply in the hemolymph.

INTRODUCTION

Lobsters (*Homarus americanus* Milne-Edwards) commonly carry microorganisms in their hemolymph (Rabin, 1965; Stewart et al., 1966a; Cornick and Stewart, 1966). Despite this fact the only recorded diseases of lobsters are an external infection named "shell disease" (Hess, 1937), and a fatal septicemia, known as "gaffkaemia" (Roskam, 1957) caused by *Gaffkya homari* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947). *Gaffkya homari*, therefore, is unique in its ability to overcome the otherwise highly effective intrinsic defence mechanisms of the lobster.

The following non-specific defence mechanisms are encountered in the hemolymph of invertebrates generally: (1) bactericidal activity (Shirodkar et al., 1958; Rabin and Bang, 1964; Rabin, 1965); (2) agglutinins (Tyler and Metz, 1945; Tyler and Scheer, 1945; Sinderman and Mairs, 1959; Cushing et al., 1963; Cohen et al., 1965); phagocytosis (Tait and Gunn, 1918; Stauber, 1950; and Tripp, 1960). In this work, the interaction of *G. homari* with these mechanisms has been compared with that of other bacteria. The results provide a partial explanation for the pathogenicity of *G. homari*.

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MATERIALS AND METHODS

Lobsters

Freshly captured lobsters were obtained from Pugwash (average weight, 337 g) or Eastern Passage (average weight, 554 g), Nova Scotia. The chelae were immobilized with rubber bands (Wilder and McLeese, 1961) and the lobsters were held, prior to experimental work, at ambient temperatures ranging from 1 to 4 C, depending upon the season. Individual shelters were provided in all tanks. All experimental work was carried out in tanks of recirculating filtered sea water (Stewart and Power, 1965) at a temperature of 15 ± 0.5 C. All lobsters were acclimated to this temperature for 2–3 weeks prior to experimentation.

Bacteria

Cultures of the bacteria were obtained from either the American Type Culture Collection (ATCC) or from a collection of bacteria isolated from marine sources and maintained at this laboratory. A strain of *G. homari* of proven virulence originally isolated from a diseased lobster was supplied by Dr H. Rabin.

Infective Cell Suspensions

The virulence of *G. homari* (Rabin) was maintained by continuous passage through lobsters held at 15 C. For experimental purposes, a volume of 0.2 ml of blood was removed from an infected lobster, inoculated into Trypticase Soy broth (TS) (Baltimore Biological Laboratory, Baltimore, Md.), and incubated for 24 hr at 28 C. A washed cell suspension in sterile 3% NaCl solution was prepared from this culture and standardized to 0.2 optical density on a Spectronic 20 colorimeter (Bausch and Lomb Inc., Rochester, N.Y.) at 420 mμ. Viable numbers were determined by the standard plate count method.

Fluorescent Labeled Suspensions

Bacteria for phagocytic studies were prepared by growing the cultures for 48 hr on TS agar containing 0.15% of the disodium salt of 4,4′-bis [4-anilino-6-bis-(2-hydroxyethyl)-amino-*s*-triazin-2-ylamino]-2-2′-stilbenedisulfonic acid (American Cyanamid Co., Bound Brook, N.J.) according to the method of Darken (1962). The washed cells were suspended in 3% NaCl solution for use. Suspensions of killed cells were prepared by the addition of formalin to a final concentration of 1.0% prior to washing and resuspending.

Hemolymph Serum

Serum was prepared from clotted hemolymph as described previously (Stewart et al., 1966b), sterilized by membrane (0.45 μ pore size) filtration (Millipore Filter Corporation, Bedford, Mass.) and stored at 4 C for use within 7 days. Protein was determined using a modified biuret method (Colowick and Kaplan, 1957).

Rabbit Red Blood Cell Suspension

This consisted of freshly drawn cells which had been washed twice in 3% NaCl and resuspended to a concentration of 5% (v/v).

Tissue Sections

Frozen sections of heart, gill, and liver tissue embedded in Optical Cutting Temperature Lab Tek (O.C.T.) (Lab-Tek, Westmount, Ill.) and cut to 8-μ thicknesses using a Model CTD Cryostat (International Equipment Co., Needham Heights, Mass.) were permanently mounted with a non-fluorescent mounting fluid (transparent nail polish). These unstained sections were examined at 440× magnification with bright field and ultraviolet illumination using a Spencer Fluorolume 645, with exciter filters A0693 and A0702 and barrier filter A0706 (American Optical Co., Instrument Division, Buffalo, N.Y.). Paraffin sections fixed in buffered formalin were cut to 8-μ thicknesses and cleared in tetrahydrofuran (British Drug Houses (Canada) Ltd., Toronto, Ont.) prior to Gram staining by the modified method of Lillie (Davenport, 1960).

Hemocyte Extracts

Extracts were prepared by grinding intact, washed hemocytes (Stewart et al., 1966b) in a Potter–Elvehjem tissue grinder with a volume of 3% NaCl solution equal to $\frac{1}{6}$ volume of hemolymph at a temperature of 4 C. The mixture was centrifuged at 12,000 × g for 15 min and the supernatant solution sterilized by membrane (0.45 μ pore size) filtration.

Bactericidal Activity

Bacterial suspensions of *G. homari* (ATCC 10400), *Achromobacter thalassius* (ATCC 14407), *Micrococcus sedentarius* (ATCC 14392), *Pseudomonas perfectomarinus* (ATCC 14405), *Gaffkya tetragena* (ATCC 10875), *Flavobacterium marinum* (FRB 140), *Bacillus subtilis* (FRB 161), and *Micrococcus conglomeratus* (FRB 185) were prepared in sterile 3% NaCl solutions and were standardized as outlined previously for the infective cell suspensions. One drop (0.025 ml) of each bacterial suspension was added with a calibrated dropping pipette (Cooke Engineering Co., Medical Research Division, Alexandria, Va.) to 4.0 ml of sterile lobster serum and to controls containing 3% NaCl with 0.05% peptone added to maintain viability. The mixtures were incubated at 15 C and mixed vigorously just prior to sampling. The numbers of viable bacteria were determined for these samples by the method of Miles and Misra (1938). Bacterial counts were repeated, increasing the agitation to disrupt any clumps caused by agglutination in the serum by including glass beads and 1% Tween 80 (polyoxyethylene sorbitan monooleate) in the diluting solution prior to plating. In addition, a similar experiment was run using heat-inactivated hemolymph serum to determine the role of the agglutinin in growth inhibition.

The survival of *A. thalassius*, *M. sedentarius*, *M. conglomeratus*, and *G. tetragena* in the lobster in vivo at 15 C was determined. Suspensions (1 ml) of these bacteria (concentration 1×10^7 bacteria/ml in 3% NaCl solution) per kg body weight were injected into the ventral abdominal sinus. Thirty days later attempts to reisolate these organisms were made by plating the hemolymph from these lobsters on TS agar.

Phagocytosis

Rabbit red blood cell suspension (1 ml) was injected into the ventral abdominal sinus of five lobsters. The hearts were excised after 5-, 10-, 15-, 30-, and 60-min intervals. Macerated cardiac tissue and hemolymph samples prepared from both injected and control lobsters were examined microscopically for phagocytosis.

The ability of lobster hemocytes to phagocytize standardized suspensions of both live and killed bacteria was investigated in a second set of experiments. The use of fluorescent labelling of the bacteria overcame the confusion arising from the similarity between hemocyte granules and cocci. Labelled bacteria fluoresced a bright blue color and natural tissue a pale green to yellow under ultraviolet light.

To determine if the hemocyte contents inhibited *G. homari* after phagocytosis occurred, a washed cell suspension of this microorganism was added to dilutions of a freshly prepared hemocyte extract at 28 C. Total bacterial numbers were determined at intervals. A solution of 3% NaCl containing 0.05% peptone was used as the control.

Hemocyte Clearance from the Hemolymph after Injection

Hemocyte counts were determined by the method of Stewart et al. (1967) after the injection of bacteria as well as after the injection of particles in the form of paraffin emulsions (Tait and Gunn, 1918), to determine if phagocytosis resulted in the clearing of hemocytes from the circulation.

Agglutination

Standard tube agglutination tests (Carpenter, 1960) were performed on freshly prepared serum. A 24-hr culture of *Brevibacterium* sp. originally isolated from lobster hemolymph (Cornick and Stewart, 1966) was used to prepare suspensions in 3% NaCl solution which were standardized to correspond with the McFarland turbidity scale 2 (Kabat and Mayer, 1961). A *Brevibacterium* sp. suspension was chosen as the standard for all agglutination tests because lobster hemolymph serum showed the highest titer against this organism. The tubes were incubated at 15 C for 48 hr or 28 C for 24 hr to determine the titer.

Tests requiring inactivation of the agglutinating principle in the hemolymph serum involved heating the serum at 56 C for 1 hr and removing the precipitate by centrifugation. The serum was then tested for the presence of agglutinin by the standard agglutination test.

The serum was tested also to determine if the agglutinin(s) could be removed by adsorption. Serum was adsorbed three times with *F. marinum* (2% packed cell volume) at 25 C; the bacteria removed by centrifugation and membrane filtration; and the serum tested for its ability to agglutinate and inhibit the growth of representative bacteria.

Serum was dialysed against three changes of artificial seawater solution containing 0.05 M tris buffer (tris (hydroxymethyl) amino methane) (THAM, Fisher Scientific Co., Montreal, Quebec), to determine if the agglutinating principle was dialysable. The ability of the agglutinin(s) to withstand freezing was tested as well. Pooled lobster sera were dispensed in sealed vials and stored at −20 C. The serum was removed and tested for agglutinating titer at various intervals up to 28 days.

RESULTS

Dose Response

The most striking result of this study (Fig. 1) was the extremely small number of bacteria required to produce a fatal infection at 15 C. A dose as low as 1×10^1/kg body wt (approximately five bacteria per lobster) killed 9/10 lobsters in 17 days. The 10th lobster was still alive after 31 days and a microscopic examination of the blood showed no *G. homari* present. The mean time to death (MTD) remained approximately constant regardless of the dose administered. Although the calculated line of best fit (Fig. 1) for dose vs. MTD had a slight slope, it was not significant according to the calculated *t*-test (Snedecor, 1956). Two lobsters receiving the highest dose,

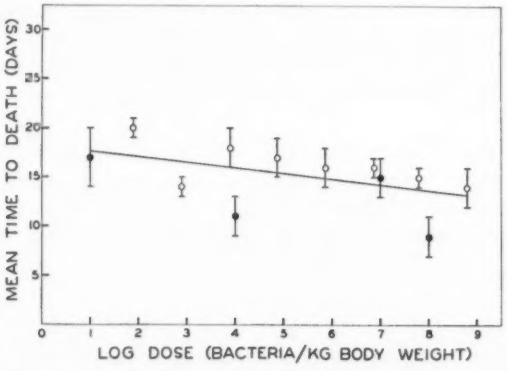

FIG. 1. Dose response relationship at 15 C. Mean times to death ±SE for 10 lobsters (5 of each sex) per dose level injected with 1 ml of different dilutions of virulent *G. homari* per kg body weight. Doses of 1×10^1 and 6×10^8 represent means of nine and eight lobsters respectively. Regression coefficient of the calculated regression line, $b = 0.571$. Symbols: ●, Pugwash lobsters weighing 210–465 g; ○, Eastern Passage lobsters weighing 404–704 g.

6×10^8/kg body wt, died after only 24 hr but no bacteria (or hemocytes) were observed in the hemolymph, and they were therefore excluded for purposes of calculating the MTD.

There was no difference in the MTD between sexes. Only 1 out of a total of 70 control lobsters died during the study and this was partially eaten by other lobsters and thus was unavailable for examination.

Bactericidal Activity of Lobster Hemolymph Serum

The bacterial numbers in the 0.05% peptone control systems remained unchanged over the entire 72-hr incubation period and consequently have been omitted from Fig. 2 and Table I. The numbers of viable *P. perfectomarinus*,

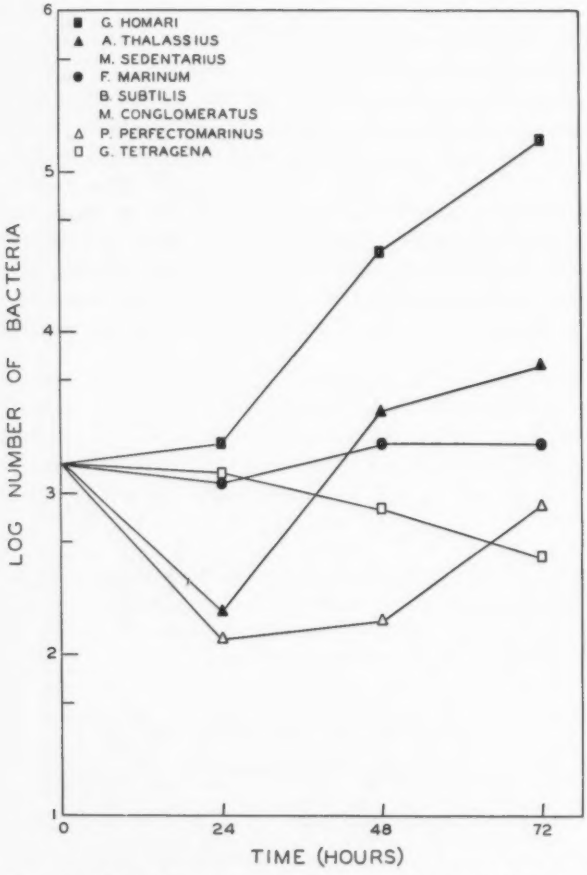

Fig. 2. Effect of untreated lobster serum on the growth of various bacteria at 15 C in vitro.

TABLE I. In vitro growth of microorganisms at 15 C in inactivated[a] hemolymph serum.

Microorganism	Incubation time (*hr*)			
	0	24	48	72
Achromobacter thalassius	1.3×10^{3}[b]	6.4×10^{4}	$>2.0\times10^{6}$	$>2.0\times10^{6}$
Gaffkya homari	2.3×10^{3}	4.8×10^{3}	5.2×10^{4}	6.0×10^{5}
Pseudomonas perfectomarinus	2.0×10^{3}	8.8×10^{3}	2.0×10^{6}	$>2.0\times10^{6}$
Flavobacterium marinum	3.6×10^{3}	4.0×10^{2}	9.2×10^{2}	8.8×10^{5}

[a]Heated at 56 C for 1 hr.
[b]Numbers of viable organisms.

A. thalassius, and *M. sedentarius* were reduced 10-fold during the first 24 hr in the serum (Fig. 2) but this effect was overcome since by the 72-hr measurement they had returned to approximately their original numbers. *Gaffkya homari* was unique in that its growth was stimulated by the serum. When tests were run using Tween 80, glass beads, and increased agitation to ensure that the formation of clumps was not causing the apparent reduction in the numbers, the results were essentially the same as those illustrated in Fig. 2.

In contrast, when heat inactivated serum was used in a similar experiment (Table I) the reduction in numbers previously observed in measurements made at the 24-hr interval was eliminated for *A. thalassius* and *P. perfectomarinus*. *Gaffkya homari* grew as well in the heated serum as in the untreated normal serum. *Flavobacterium marinum* showed increased growth toward the end of the incubation period. Agglutination tests using this heated serum with appropriate controls against *Brevibacterium* sp. were uniformly negative.

Adsorption of the serum with *F. marinum* appeared to remove the growth-inhibiting factors only. *Flavobacterium marinum*, *P. perfectomarinus*, and *G. homari* grew as well in the adsorbed serum as *G. homari* did in the untreated serum.

Re-isolation of *A. thalassius*, *M. sedentarius*, *M. conglomeratus*, and *G. tetragena* 30 days after suspensions of these organisms had been injected into lobsters (groups of 10 animals) at 15 C could not be accomplished. Apparently the hemolymph defense factors had removed or eliminated all of these organisms in vivo.

PHAGOCYTOSIS IN TISSUES

A microscopic examination of cardiac hemolymph after injection of rabbit red blood cells (Fig. 3) showed that phagocytosis occurred within 15 min of the injection. The numbers of hemocytes were markedly reduced. The lobsters were killed after 1 hr and the excised hearts were red in color as a result of masses of red blood cells contained in the cardiac tissue.

The injection of both live and killed fluorescent labelled cells of *G. homari* and *B. subtilis* resulted in a reduction in circulating hemocyte numbers after

15 min but no phagocytized bacteria were observed in moist hemolymph mounts. These labelled cells, however, were observed fluorescing within cells located in frozen heart, liver, and gill tissue sections when these were examined microscopically under ultraviolet light. The corresponding Gram-stained sections of cardiac tissue (Fig. 4*a* and *b*) showed masses of cells similar to hemocytes which contained the bacteria and were closely associated with the connective tissue cells.

Lobsters infected for extended periods before death (approximately 3 weeks or longer) contained numerous tiny black specks in the gill raches, swimmerettes, and ventral abdominal sinuses. Microscopic examination of tissue sections from gills containing these nodules (Fig. 5*a*) showed that they were composed of aggregates of tightly clumped tissue cells containing a reddish-brown material which is as yet unidentified. A section through one of these nodules (Fig. 5*b*) showed numerous *G. homari* inside.

Phagocytic Activity

Phagocytic activity is expressed as the mean number of bacteria per phagocyte observed in a random selection of microscopic fields containing phagocyte aggregates in tissue sections. In general, phagocytic activity was low (Table II), with a mean of only one to three bacteria observed per phagocyte. Phagocytes contained an average of two to three *G. homari*, compared with only one *B. subtilis* per phagocyte, which was significantly less, regardless of the tissue involved or whether live or killed bacteria were injected. There was no difference between phagocytic activity of heart or liver tissue for *B. subtilis* but phagocytes associated with heart tissue contained an average of three cells of *G. homari*, significantly more than in liver tissue (two per phagocyte).

Table II. Phagocytic activity[a] of lobster hemocytes in heart and liver tissue.

	Heart tissue		Liver tissue	
Microorganism	Live suspension	Killed suspension	Live suspension	Killed suspension
Gaffkya homari	3±0.3(34)	3±0.2(239)	2±0.1(104)	2±0.3(94)
Bacillus subtilis	1±0.02(402)	1±0.1(65)	1±0.1(48)	1±0.1(128)

[a]Mean number of bacteria per phagocyte followed by standard error of the mean with the number of phagocytes counted in parentheses. Random selection of microscopic fields containing phagocyte aggregates in tissue. In some cases the entire section was examined.

Growth in Hemocyte Extracts

Hemocyte extract was neither bactericidal nor bacteriostatic to a dilute suspension of *G. homari* (Fig. 6). The bacterium grew better in the extract than in the control system, and the amount of growth was related to the concentration of the extract expressed in terms of the protein concentration.

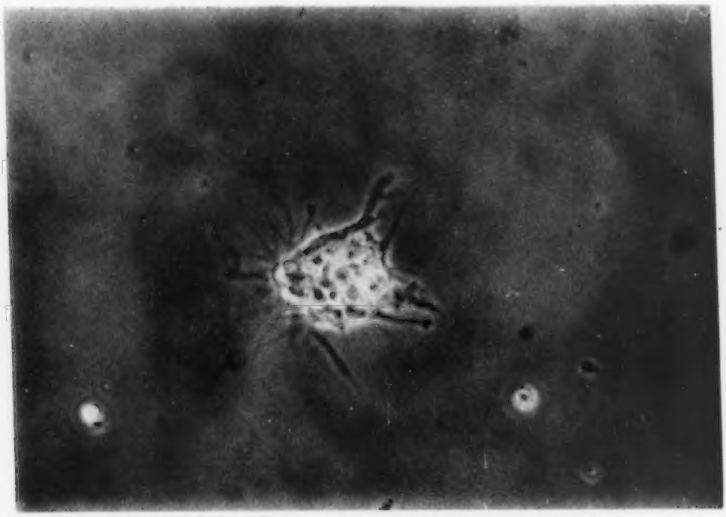

FIG. 3. Hemocyte containing rabbit red blood cells. Phase contrast ×1000.

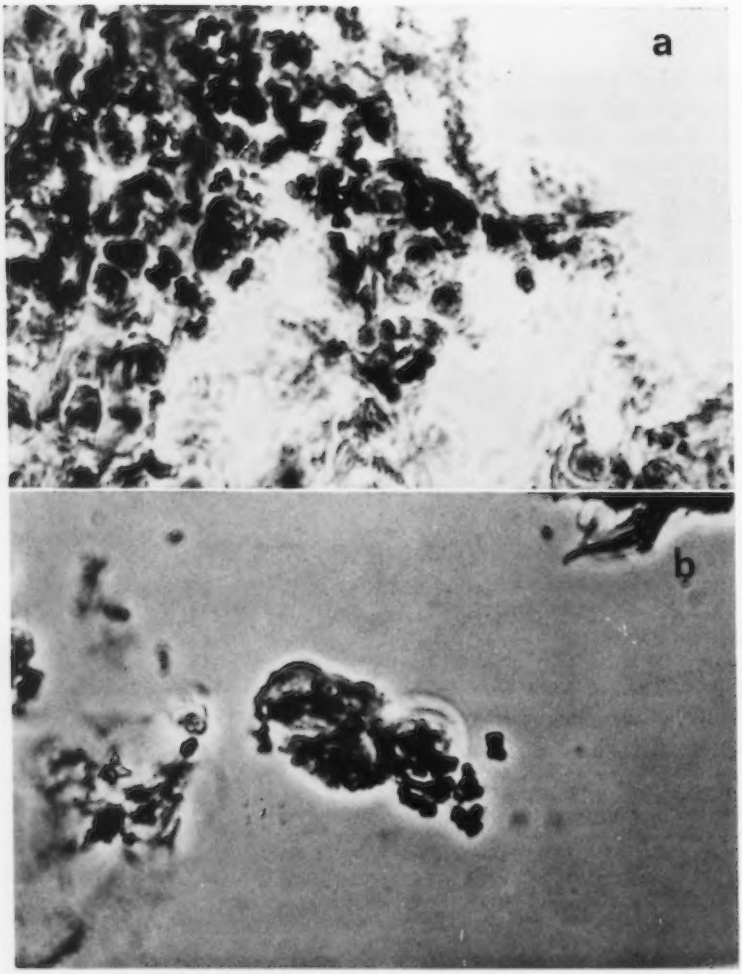

FIG. 4. *a*, Section through heart of lobster infected with *G. homari*. Gram tissue stain ×1200. *b*, Isolated cells in heart tissue containing *G. homari*. Gram tissue stain ×1200.

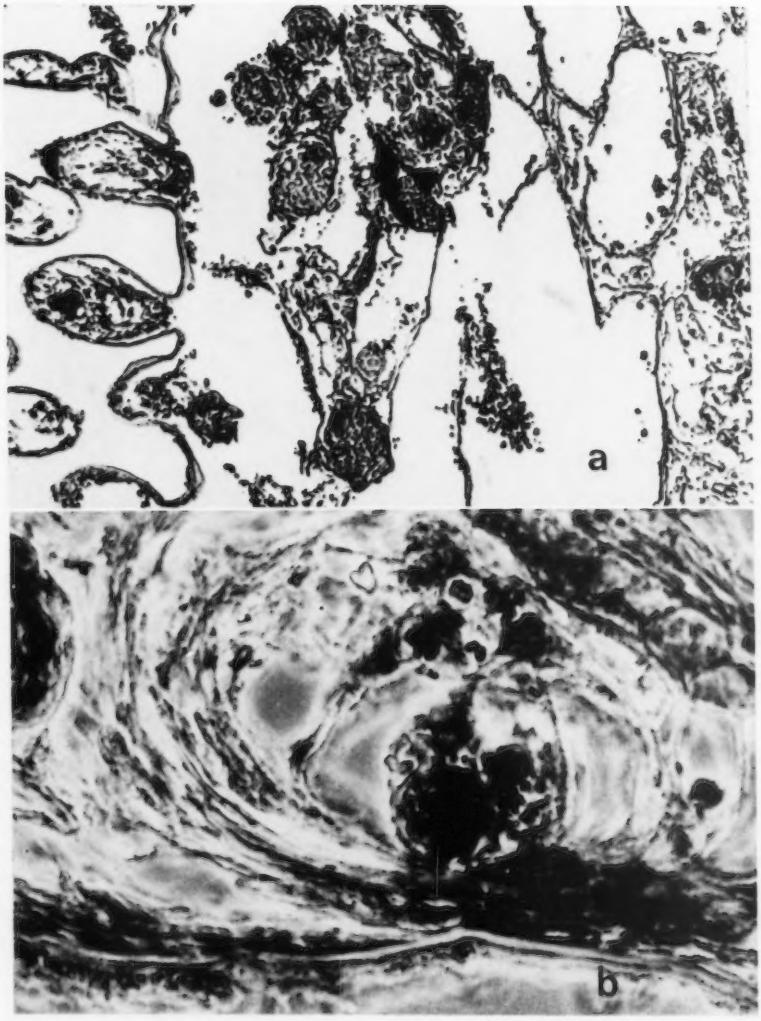

FIG. 5. *a*, Section through gill of lobster infected with *G. homari* showing nodules of tightly packed cells. Gram tissue stain ×120. *b*, Enlarged view of Fig. 5*a* showing small nodule of cells containing *G. homari*. Gram tissue stain ×1200.

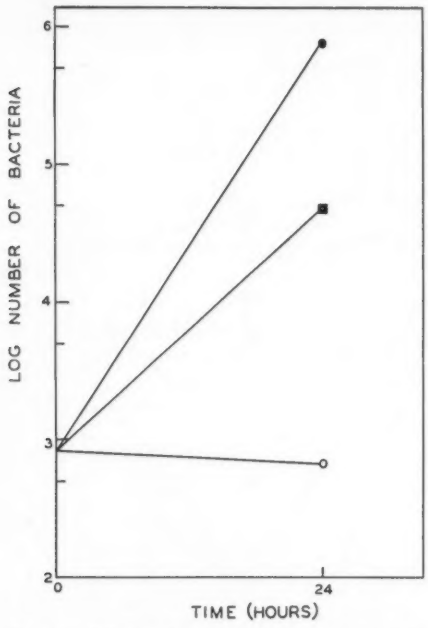

FIG. 6. Effect of hemocyte extract on the growth of *G. homari* in vitro at 28 C. Symbols: ●, native extract (protein content 7.5 mg/ml); ■, ½ concentration of extract; ○, no extract.

EFFECT OF BACTERIAL INJECTIONS ON HEMOCYTE COUNTS

Lobsters injected with bacteria (*B. subtilis* and *G. homari*) exhibited a significant reduction in circulating hemocyte numbers after only 15 min (Fig. 7), whereas those receiving the paraffin emulsion or artificial seawater solution showed slight to no reduction. Bacterial suspensions in paraffin emulsion resulted in a reduced rate of clearance of hemocytes. The results indicate a definite relationship between active phagocytosis and hemocyte clearance after injection of particulate matter. Hemocyte levels returned to normal 5 hr after *G. homari* injections but recovery was much slower with *B. subtilis*. Hemocyte counts over an extended period (Table III) showed that after the hemocytes had returned to initial levels, their levels tended to fluctuate for a period of at least 24 hr.

AGGLUTINATION OF BACTERIA

The natural agglutinin in lobster serum (Table IV) was active against suspensions of all the bacteria tested with the notable exception of all strains of *G. homari*. Adsorption of the serum with *F. marinum* removed the agglutinin for all bacteria tested with one exception, *Brevibacterium* sp., where a reduced titer only was noted. The titer was generally low, varied with the individual

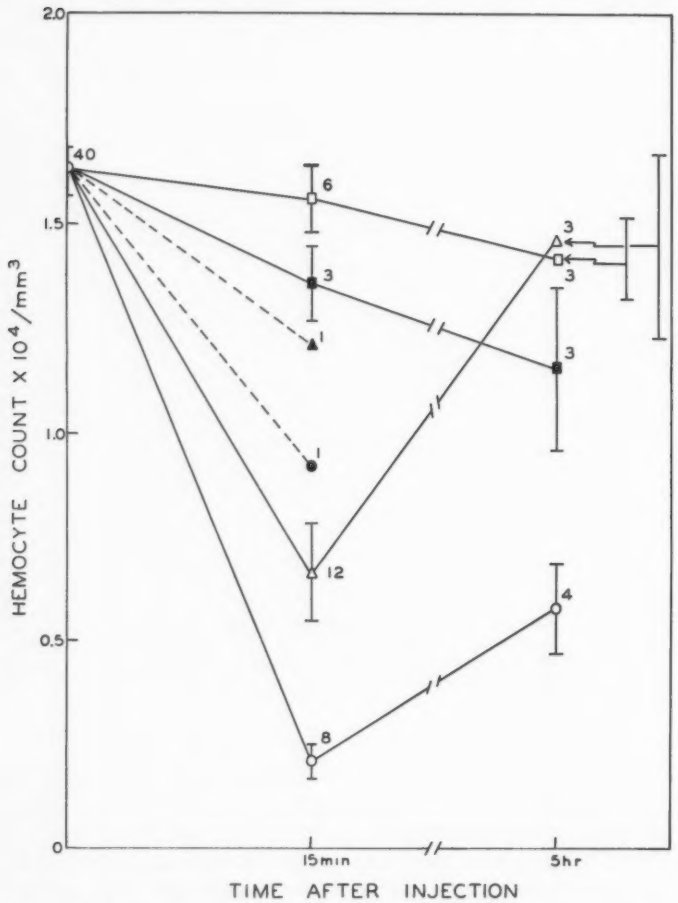

FIG. 7. Effect of injections (into lobsters) on hemocyte counts. Mean number of hemocytes, SE and the number of lobsters sampled. Symbols: □, artificial sea water; ■, paraffin emulsion; ○, *B. subtilis*; △, *G. homari*; ▲, G. *homari* in paraffin emulsion; ●, *B. subtilis* in paraffin emulsion.

lobster (Table V), and was unrelated to the sex of the lobster or the protein content of the serum.

A preliminary characterization of the agglutinating principle revealed that it was non-dialysable and was inactivated by heating for 1 hr at 56 C. In addition, the agglutinin titer was unchanged after 24 hr at −20 C. Activity was reduced by the 6th day; however, some activity was still present after 28 days.

TABLE III. Effect of bacterial injections on total hemocyte counts.[a]

Material injected	Hemocytes/mm³ × 10⁴		
	0	6 hr	24 hr
ASW	2.1±0.2(5)	2.9±0.2(5)	2.1±0.3(5)
Bacillus subtilis	3.2±0.5(5)	2.3±0.4(5)	1.4±0.2(5)
Gaffkya homari	2.1±0.3(5)	2.8±0.3(5)	1.5±0.2(5)

[a]Average values are given followed by the standard error of the mean with the number of lobsters sampled in parentheses.

TABLE IV. Lobster serum[a] agglutinin titers at 15 C.

Bacterium	Source	Agglutinin	Titer[b]	
Normal serum				
Achromobacter thalassius	ATCC 14407	+	4	
Bacillis subtilis	FRB 161	+	8	
Brevibacterium sp.	FRB 27	+	4	
Flavobacterium marinum	FRB 140	+	4	
Gaffkya homari	Dr. Rabin	−	0	
" "	ATCC 10400	−	0	
" "	FRB 88B	−	0	
" "	FRB 37R	−	0	
Gaffkya tetragena	ATCC 10875	+	8	
Micrococcus conglomeratus	FRB 185	+	8	
Micrococcus sedentarius	ATCC 14392	+	2	
Pseudomonas perfectomarinus	ATCC 14405	+	2	
Normal serum vs. serum adsorbed with *Flavobacterium marinum*				
Flavobacterium marinum	FRB 140	+	16	N.S.[c]
		−	0	A.S.
Achromobacter thalassius	ATCC 14407	+	2	N.S.
		−	0	A.S.
Micrococcus conglomeratus	FRB 185	+	2	N.S.
		−	0	A.S.
Brevibacterium sp.	FRB 27	+	16	N.S.
		+	8	A.S.

[a]Pooled sera (protein concentration 37.8 mg/ml).
[b]Reciprocal of serum dilution.
[c]Normal serum, N.S.; serum adsorbed with *F. marinum*, A.S.

DISCUSSION

The method of counting as well as injecting very small numbers of bacteria into an animal involves a source of error. Within these limits of error, however, the results indicate that numbers of *G. homari* approaching a single bacterium are sufficient to produce a fatal infection in the lobster at 15 C. These results confirm the earlier observations of Rabin (1965) that as few as 10^3 bacteria proved fatal, and emphasize the lobsters' complete lack of defense against this pathogen.

TABLE V. Agglutination titers from sera of individual lobster.[a]

Lobster number	Sex	Serum protein (*mg/ml*)	Agglutination titer[b]
1	M	28.0	64
2	M	52.0	64
3	M	–	128
4	F	32.0	512
5	F	41.0	32
6	F	–	256

[a]Test microorganism, *Brevibacterium* sp. at 28 C.
[b]Reciprocal of serum dilution.

Toxin production by *G. homari* has not been demonstrated successfully. The prolonged time to death (TD = 17 days) after infection, the great variation in TD between individual lobsters at any one dose level (Fig. 1), and the symptom of gradual weakening prior to death all suggest that pathogenicity is due to depletion of body reserves as would be expected to occur in a true septicemia.

One of the more important defense mechanisms possessed by the lobster is the inhibitory activity of the hemolymph. Rabin (1965) observed that the hemolymph serum of the lobster inhibited the growth of *Vibrio* sp. in vitro. Our results indicate that this principle is also effective against members of the genera *Pseudomonas*, *Achromobacter*, and *Micrococcus*. Activity was not specific for all the bacteria tested. *Gaffkya homari* was not only unaffected by the serum bactericidal principle but its growth was greatly stimulated by the serum, an important factor enhancing pathogenicity.

The fact that heat treatment removed both the agglutinating and growth-inhibiting activities of the serum suggests that either this is a single principle or that the bactericidal principle is inactivated at a temperature below that required to destroy the agglutinin.

Serum was inhibitory in vitro for *A. thalassius* and *M. sedentarius* for the initial 24-hr period; this effect was overcome after 72 hr. When these bacteria were injected into lobsters at the same temperature (15 C), they could not be isolated from the hemolymph 30 days later, indicating that the reduction in inhibitory activity after 72 hr in the in vitro test was related to test conditions.

In decapods, hemocytes which have phagocytized foreign particles accumulate in capillary and lacunar networks and may result in reductions in hemocyte numbers (Maynard, 1960). In our studies rabbit red blood cells were phagocytized actively, apparently in the hemolymph, and then accumulated in the cardiac tissue with a corresponding decrease in the number of circulating hemocytes. These results are similar to those obtained by Bang (1956) who observed reduced circulating hemocyte numbers and hemocyte

accumulation in the tissues in the horseshoe crab, *Limulus polyphemus*, injected with a Gram negative bacterium. Caution should be exercised, however, in associating reductions in hemocyte numbers with phagocytosis since our unpublished observations indicate that hemocyte counts can fluctuate widely, depending upon a number of factors.

Pathogenicity in vertebrates is commonly related to the inability of phagocytes to effectively engulf encapsulated pathogens (Carpenter, 1956). The capsules surrounding groups of *G. homari* in the blood of infected lobsters stain deeply with polysaccharide specific Alcian Blue stain (McKinney, 1953). Our experiments have shown that *G. homari* grown on laboratory medium were actively phagocytized. The development of capsules in vivo at a later stage in the infection may reduce the phagocytic activity and thus enhance multiplication of the pathogen. Although phagocytosis does occur, *G. homari* is apparently not destroyed within the phagocyte. This is substantiated by the fact that very low doses are successful in establishing fatal infections.

The aggregates of bacteria-laden cells observed in sections of gill tissue taken from infected lobsters are not uncommon. Groups of phagocytes occur in many crustacean species and chiefly in the gill raches (Parry, 1960).

Tyler and Metz (1945) have reported species specific hemagglutinins in spiny lobster (*Panulirus interruptus*) serum. This is the first report of agglutinin(s) activity against bacteria in lobster (*H. americanus*) serum and establishes its importance as an integral part of the lobster's defense mechanism. The fact that of all the bacteria tested, only the strains of *G. homari* were not agglutinated by lobster serum is a highly significant factor contributing to its pathogenicity. The fact that heat inactivation of the serum or adsorption with *F. marinum* removes the growth-inhibiting effect of the lobster hemolymph raises the possibility that the inhibiting effects of the serum upon growth may be caused by the agglutinin(s). It is also possible that more than one agglutinin exists since adsorption with *F. marinum* only halved the titer for *Brevibacterium* sp. rather than eliminating the agglutinin effect as it did for the other organisms.

These studies show that the pathogenicity of *G. homari* for the lobster is largely due to the suitability of the hemolymph as a growth medium, coupled with the failure of the lobster's other defense mechanisms, such as agglutinin and phagocytic activities, to cope with the rapid multiplication of the pathogen.

ACKNOWLEDGMENTS

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Influence of Changing Seasons on Schooling Behavior of Yellow Perch[1]

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ABSTRACT

Analysis of echo-sounder and sonar records made on Lake Mendota during summer and winter indicated that the schooling behavior of yellow perch, *Perca flavescens* (Mitchill), changed markedly between the two seasons. The distance separating individuals in schools during summer was less than 1.5–2.0 ft, consequently producing dense traces on the recorder charts which showed no definition of individuals. During winter, however, the fish-to-fish distances often were greater than 1.5–2.0 ft, producing many traces showing the individual fishes in a school. The mean distance between the top and bottom fish in a school during winter was 22.1 ft (6.7 m); in summer only 8.5 ft (2.5 m). High correlations existed between light transmission of the water and school size ($r = .77$) and between temperature and school size ($r = -.89$). The possible adaptive significance of this seasonal change in behavior is discussed.

INTRODUCTION

A comparison of echo-sounder records made on Lake Mendota during summer and winter reveals a striking difference in the size of schools of yellow perch, *Perca flavescens* (Mitchill), between the two seasons. Although the distance between the uppermost and lowermost fishes in a school during summer will not exceed 5 m, this same distance during winter will have increased in many cases to as much as 12 m (Hergenrader and Hasler, 1966). Two factors, spacing and number of individuals, may affect the size (or, more correctly, the area) of a school of fish, either independently or together.

It has been repeatedly demonstrated (with few exceptions) that vision is necessary for the formation and maintenance of fish schools (Breder, Jr., 1959; Blaxter and Parrish, 1965). Perch in Lake Mendota do not school during darkness (Hergenrader and Hasler, 1966). One could expect that seasonal changes in water clarity might influence the internal structure and behavior of fish schools. Temperature, because it regulates the level of activity of fishes, might also produce seasonal changes in schooling behavior.

In this report we will consider the apparent influence of seasonally changing light and temperature conditions in eutrophic Lake Mendota on the schooling behavior of yellow perch, particularly as related to the size of the school in summer and winter.

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METHODS AND MATERIALS

The study was conducted entirely on Lake Mendota, Wisc., in waters ranging from 10 to 23 m in depth. Data were obtained during the winters of 1963–66 and during the summers of 1964 and 1966 with Bendix DR-9 (50 kHz) echo sounders (see Hergenrader and Hasler, 1966, for specifications). In addition, during 1966 a Minneapolis-Honeywell sonar (178 kHz) (Hergenrader and Hasler, 1967) was used in conjunction with the echo sounders to study the size and behavior of perch schools.

The echo sounders utilize dry recording paper scaled from 0 to 200 ft (0–61 m) in depth graduations of 2 ft. The pulse length of the Bendix units is equivalent to a travel time of sound in water shown on the recording chart as approximately 1.5–2.0 ft. Hence, fishes must be separated by vertical distances (when directly beneath the sounder) of approximately 1.5–2.0 ft in order to be recorded as separate individuals. Traces from fish closer together than 1.5–2.0 ft merge and appear as one wide trace.

The sonar equipment, in addition to having a graphic recorder, has an 18-cm Plan Position Indicator (PPI) output. This display gives the bearing and range of the school. Manual swinging of the sound beam back and forth across a given school causes the outline of the school to appear as a trace on the fluorescent face of the PPI. It was possible to approximate the area of the school by placing an acetate facsimile (0.01 mm thick) directly on the PPI and outlining the trace of the school with a felt-tipped pen. We assumed that the area of the trace on the PPI was directly proportional to the area (size) of the school in water.

One factor which will influence the area of the trace on the PPI is the relation of target aspect to the sound beam. When the longitudinal axis of the target is at an oblique angle to the sound beam, the trace will be slightly larger than when the longitudinal axis of the same target is perpendicular to the beam. The distance from the target also appears to have some effect on the area of the trace in that very close targets produce traces that are smaller than those at extreme ranges. We assumed that in recording the areas of 475 schools the target aspect was a random variable, particularly since schools of perch in Lake Mendota show no preferred swimming direction (Hergenrader and Hasler, 1967). Similarly, the mean distances at which the initial recordings of the schools were made differed very little between winter and summer. By recording during both summer and winter periods with the echo sounder and sonar, comparable data on the size and behavior of schools at different seasons were obtained.

The area of a given school was determined by placing a millimeter grid on the acetate facsimile and counting the squares covered by the trace. The vertical extent and "cohesiveness" of a school was determined solely from the echo-sounder charts.

Operations during summer were conducted from a pontoon barge and during winter directly on the ice itself. Data were collected at approximately

weekly intervals, and during any one recording session we generally remained on station throughout the duration of daylight.

The relative clarity of the water was determined with a Whitney underwater photometer which utilized a Weston photronic cell (Model 856 RR) as the sensor. The quantity of light at each meter of depth was measured and from these data the mean light transmission of the water column was determined. All measurements were made at the same time of day and under similar sky conditions. Profiles of water temperature were made with a Whitney thermistor thermometer.

The methods for identifying specific traces on the echo sounder have been described previously (Hergenrader and Hasler, 1966, 1967). Briefly, we believe that we were primarily recording yellow perch with our equipment because of their relative abundance in the lake (17 perch to every white bass, *Roccus chrysops* (Rafinesque), probably their nearest rival in numbers present in the pelagic region of the lake (Tibbles, MS, 1956)) but more especially because in 95 instances we captured and identified the fish producing specific traces on the Bendix chart. Yellow perch accounted for 91 of these traces whereas the remainder consisted of 2 white bass and 2 bluegills, *Lepomis macrochirus* Rafinesque.

INTERPRETATION OF TRACES

Schools of perch during winter (Fig. 1, 2) not only produced traces which persisted for a longer time than those made during summer (Fig. 3, 4) but also extended vertically a much greater distance. The long traces result partially from the fish swimming more slowly at the low water temperatures during winter (Hergenrader and Hasler, 1967), thus remaining in the sound field of the recorder for a longer time, and they also may be longer because there may be a greater number of fish present in winter schools. We cannot resolve this problem with our present information. However, because of the relatively shallow depth of Lake Mendota, the vertical extent of the school can only be a reflection of the distance between the uppermost and lowermost fishes in a school (more accurately, a measure of the difference in distance of the uppermost fishes in the school from the transducer of the echo sounder.) This could result both from more fishes being present in a school and because the individuals in a school maintain greater fish-to-fish distances during winter than during summer. Figures 1 and 2 indicate that wider spacing accounts for at least a part of the difference. In almost every case the trace has a considerable amount of background grey showing through the black. This probably results from a decrease in the strength of the echo which in turn is decreased because the individuals in the school are more widely spaced. In many instances the discrete traces of individual fish in the schools can be seen. This means that the vertical distance between individuals is at least as great as the pulse length of the machine (1.5–2.0 ft). If this were not so, the traces of individuals would merge and appear as one wide trace.

Records of schools made during summer (Fig. 3, 4) show no discrete traces of individuals within a school; rather, there is simply one wide trace and there is no background grey showing through. This is to be expected in view of the fact that the separation distances between perch in summer schools vary from 16 cm (Hasler and Bardach, 1949) to 20–25 cm (Hasler and Villemonte, 1953), distances much less than the pulse length of the Bendix sounder.

The average distance between the top and bottom fish in a school, from randomly selected records of 16 schools recorded during winter, was 22.1 ft (6.7 m). During summer, this same distance was 8.5 ft (2.5 m). The standard deviations for these means were $s = 9.8$ and $s = 1.7$ respectively.

Analysis of the areas of perch schools drawn on the acetate facsimiles of the PPI of the sonar (Fig. 5) also indicated that schools of perch during winter occupy a much larger area than during summer. Furthermore, the differences in areas of schools between the two seasons agree well with the information on school size derived from the echo-sounder charts.

We suggested in the introduction that because vision is important in schooling, water clarity, as evidenced by the light transmission characteristics, could perhaps influence schooling behavior and school structure. The coefficient of correlation between the light transmission of the water and the area of the school (Fig. 6) was 0.77, indicating that there is a rather strong, direct relationship between light transmission and school size. In winter, the waters of Lake Mendota are usually quite clear. During summer, however, the high seston content reduces visibility and light transmission markedly.

That the seasonal change in temperature might influence schooling is borne out in that the correlation between mean water temperature and the area of the school was highly negative ($r = -.89$).

DISCUSSION

Even with greatly increased water clarity during winter, the relation between clarity and school size would be meaningless if the quantity of light which reached a given depth was insufficient for the perch to see each other. In spite of the fact that, in winter, Lake Mendota is covered by a layer of ice that usually reaches a thickness of some 50 cm, the quantity of light below 10 m (our shallowest station) exceeds the quantity below this depth during summer (Hergenrader, MS, 1967), average values of 20–25 ft-c not being uncommon. This is well above the threshold value for photopic vision in teleosts. Thus, fish under light conditions extant in winter could maintain greater fish-to-fish distances within a given school than during summer. Our data indicate that they do this.

A more fundamental inquiry concerns the significance of such behavior. The answer to this question is not clear but one possibility is that it is associated with food distribution and feeding. One of the major winter food items of yellow perch in Lake Mendota is zooplankton (Hergenrader, 1967). If this food supply exhibits a patchy distribution, as zooplankton populations

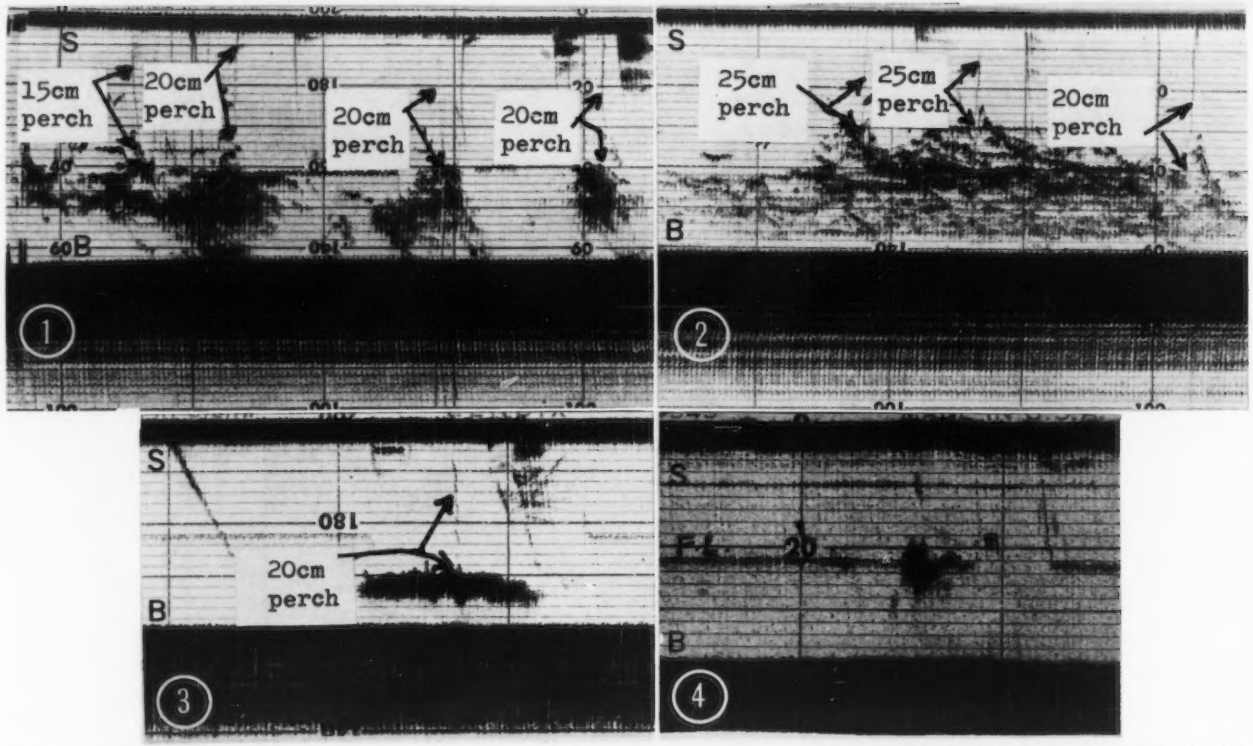
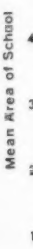


FIG. 1. Echo-sounder traces of yellow perch schools recorded during February 1966. Depth of water 63 ft (19 m), time 1028–1045 hr. The arrows indicate where perch were caught and pulled to the surface. Note that the discrete traces of individuals may be seen within the schools. S = surface, B = bottom.

FIG. 2. Record of perch school, February 1966. Depth 60 ft (18 m). The arrows indicate where perch were caught and pulled to the surface. The traces of some individuals in the school are apparent. S = surface, B = bottom.

FIG. 3. Record of perch school, August 1966. Depth 40 ft (12 m), time 0928–0937 hr. Arrows indicate where a perch was caught and pulled to the surface. No discrete traces of individuals within the school can be discerned. S = surface, B = bottom.

FIG. 4. Record of school of fish (probably perch), August 1966. Depth 38 ft (11.5 m), time 1057–1103 hr. S = surface, B = bottom, Fl. lead sinker of fishing line, which clearly indicates the pulse width of the Bendix sounder.


Mean Area of School

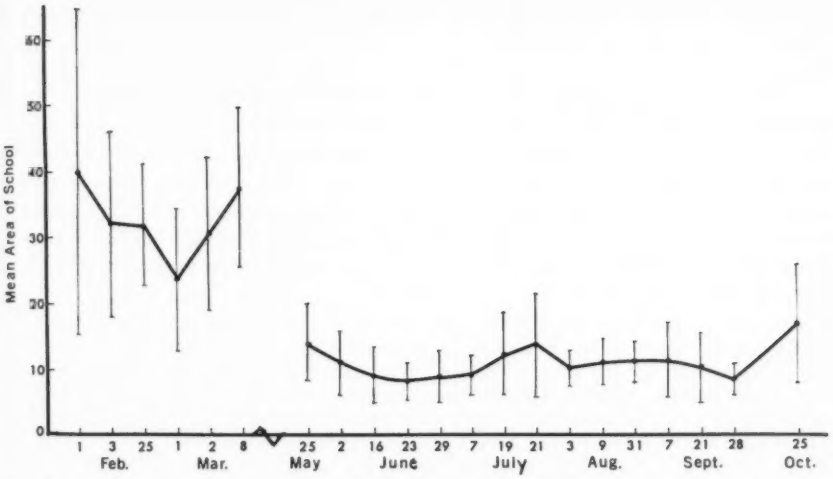


FIG. 5. Comparison of the areas (mm²) of 475 perch schools derived from acetate facsimiles of the PPI of the sonar, summer and winter, 1966. The vertical line through each point indicates one standard deviation.

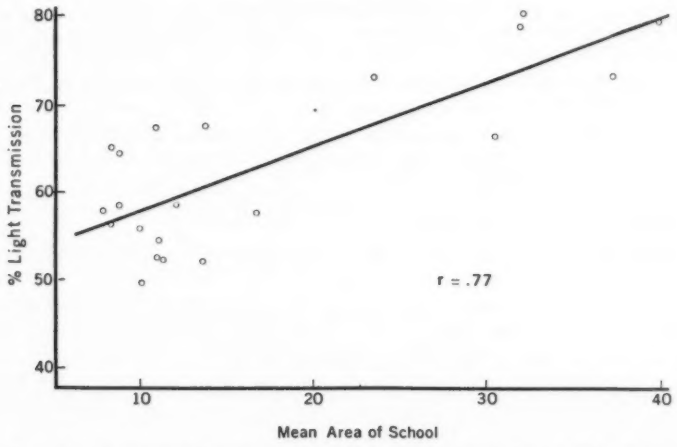


FIG. 6. The correlation between mean light transmission of the water column and the areas (mm²) of perch schools derived from the PPI facsimiles.

generally do, a school which had an increased area would scan a larger segment of the environment and might well increase the probability of encountering these zooplankton patches. This could be important in view of the fact that zooplankton abundance in Lake Mendota is generally at its lowest levels during winter. It may be, on the other hand, that the increased separation distance

during winter is a manifestation of a change in some intrinsic factor in the school (attraction or repulsion) which comes about with increasing water clarity.

The biological significance of the negative correlation between water temperature and school size is unclear. Breder and Nigrelli (1935) have shown that low water temperatures can induce non-schooling centrarchids to form compact aggregations superficially resembling schools of fish. We are not aware of any data which show that low water temperatures cause schools to become less cohesive. In the case of the yellow perch it may be that with the lower activity levels during winter there is a corresponding reduction in the intrinsic schooling drive.

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The authors wish to thank those members of the Laboratory of Limnology who assisted with the field work. The project was supported by grants from the Wisconsin Conservation Department, the Office of Naval Research (Nonr-1202(04), NR 301-903), and the National Science Foundation (G-9679, GB 3123).

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A Statistical Analysis of Ration and Growth Relationship of Plaice (*Pleuronectes platessa*)[1]

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ABSTRACT

Dawes (1930, J. Marine Biol. Assoc., **17**: 103 and 877) gave extensive data on average daily rations and fortnightly growth increments of *Pleuronectes platessa* fed on *Mytilus edulis*. The data are analyzed here in an attempt to formulate a ration and growth increment relationship. Fortnightly growth increments, Δw, were arranged in eight groups according to eight ranges of fish weights. The growth increments in each group, associated with each average daily ration level (R), gave evidence of the normal distribution. Each range of fish weights was characterized by a certain range of daily rations associated with growth increments having homogeneous variance, as well as by a certain daily ration, R_m, associated with maximum growth efficiency. There was evidence that growth increments, of each range of fish weights, were linearly related with $\sqrt{|R-R_m|}$ such that the value of the square root is positive if $R > R_m$ and negative if $R < R_m$, i.e., $\Delta w = a \pm b\sqrt{|R-R_m|}$ where a and b are parameters.

INTRODUCTION

The relation between food and growth of fish has been a subject of interest for some time. Dawes (1930a, b) gave extensive data on the subject for plaice, *Pleuronectes platessa*, during their third growth season. Paloheimo and Dickie (1966a, b) re-analyzed Dawes (1930a, b) data and many others to formulate a relationship between ration and growth in fishes. Their second analysis showed frequent deviations of experimental data from their proposed relationship, viz. $\Delta w/\Delta t = R\, e^{-a-bR}$, where Δw is growth; R is the ration intake during the period of time Δt; and a and b are parameters of the relationship. In this paper Dawes' (1930a, b) data are re-analyzed in order to derive a more general relation between ration and growth.

MATERIALS AND METHODS

Dawes' (1930a, b) "refined data" were obtained in 1929 and 1930 by feeding 101 2-year-old *P. platessa* on chunks of *Mytilus edulis*. Fish were held at Cawsand and Lympstone in separate wire-cage compartments on large floats. Feeding was carried out at two levels, viz. maintenance (minimum rations) and ad libitum (with excess food). The maintenance rations were assigned a priori for fortnightly intervals, then adjusted on basis of weight gains. Four fish were fed on intermediate rations. The daily rations for each fish were weighed and daily averages of 2-week intervals were reported. Changes in weight and length were recorded fortnightly. The 101 fish supplied about

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928 records of fortnightly growth increment. Dawes (1930a) supplied a group of six male fish and a second of six female fish with a daily ration of 0.42 g for 44 days. Dawes (1930a, Table 8) gave initial and final weights and growth increment of each fish. The averages of initial and final weights and growth increments were as follows:

Sex	Initial avg weight	Final avg weight	Growth increments
Males	16.7	18.4	4.0, 4.0, 0, −3.0, 3.0, 2.5
Females	22.1	22.6	1.5, 1.5, 1.0, 1.0, 0, −2.0

The Kolmogorov–Smirnov test (Lindgren, 1962) gave evidence of the normal distribution of growth increments of males as well as females (males: $|D_n| = 0.2739$; females: $|D_n| = 0.3122$, 5% level = 0.521). Homogeneity of variance of growth increments of males and females was evidenced by the variance ratio test ($F = 7.575/1.8 = 4.208$, $n_1 = 5$, $n_2 = 5$, 5% level = 5.05), and the nonsignificant difference between their means of growth increments was evidenced by T^2 test ($T^2 = 0.833$, df = 1, 10, 5% level = 4.96) (Lindgren, 1962). That is, males and females with a fixed ration and weighing from 16 to 23 g (about 50% difference in weight) may have the same normally distributed population of growth increments.

On such a basis, Dawes' (1930a, b) data were arranged in eight groups according to fish weights. The groups (in grams) were 15–24.9, 25–40, 40.1–60, 60.1–90, 90.1–105, 105.1–120, 120.1–150, and 150.1–211.

Ration and growth increment tables were arranged for each separate group. The daily ration intervals were 0.5 g. The fortnightly growth increment intervals for the first two groups were 0.5 g to obtain about at least 20 classes (Snedecor and Cochran 1956). Growth increment intervals of 1.0 g were found more feasible for the other groups. Tables of the fifth and sixth groups were combined together as well as the seventh and the eight. They were combined because they showed approximately similar growth increments at each ration level.

In preparing the tables, there were growth increments derived from initial and final fish weights belonging to two successive tables. Such cases were recorded in the table covering the majority of the fish weight range, otherwise disregarded. For example, the growth increment between 20- and 26-g fish was recorded in Table I, whereas the increment between 20- and 30-g fish was disregarded. This decreased the number of records from 928 to 844. Twenty extreme variates were eliminated.

SEASONS AND THE FISH GROUPS

To study the effect of seasons or temperatures on ration-growth relationship to be derived from the fish groups, a table was arranged for each group to show the distribution of average daily rations and corresponding fortnightly growth increments within the different months of the experiments. An occurrence

of an average daily ration of a fish group within any month was scored by 1. Monthly frequencies were arranged for the ration levels of the fish groups. Heterogeneity between monthly frequencies of ration levels within each fish group was studied by the chi-square method. The tables showed that frequencies in many cells were too few to allow a proper test. A number of months had to be combined in one cell or season. Investigation of temperature diagrams by Dawes (1930a, b) showed May and June with closer increasing temperatures (season 1); July, August, and September with more or less closer maximum stationary temperatures (season 2); and October, November, and December with decreasing temperatures (season 3). The ration monthly frequencies were combined into the above three seasons. To increase cell frequencies once more, the 6, 10, 13, 14, 17, and 17 ration levels of Tables I–VI were combined into 5, 9, 10, 12, 11, and 8 cells respectively. The totals of seasonal frequencies of each fish group were the following:

Fish groups	Frequencies of seasons		
	1	2	3
1	34	23	4
2	29	81	17
3	13	123	88
4	27	80	83
5	8	62	77
6	6	18	51

It was found more advantageous to combine seasons 3 and 2 in groups 1 and 2, and seasons 1 and 2 in the other groups. Chi-square test provided evidence of nonsignificant differences between seasonal distributions of ration levels within fish groups 1 and 2 (group 1: $\chi^2 = 5.203$, df = 4, $0.5 > P > 0.25$; group 2: $\chi^2 = 7.68$, df = 8, $0.75 > P > 0.5$). Fish group 3 provided evidence of homogeneity after eliminating 2.25 and 4.25 combined with 4.75 ration levels ($\chi^2 = 8.08$, df = 7, $0.5 > P > 0.25$). Group 4 provided evidence of heterogeneity ($\chi^2 = 34.06$, df = 11, $P < 0.005$) and group 5 provided evidence of homogeneity after excluding 0.25, 0.75, and 4.75 ration levels ($\chi^2 = 11.11$, df = 7, $0.25 > P > 0.1$). Group 6 gave evidence of homogeneity after excluding 1.25 ration level ($\chi^2 = 6.94$, df = 6, $0.5 > P > 0.25$). Therefore it may be concluded that seasonal effects on ration–growth relationships may have been insignificant in fish groups 1, 2, and 6, may have not been serious in groups 3 and 5, and were serious in fish group 4.

A comparison between the total seasonal distributions of the six fish groups gave evidence of heterogeneity between them ($\chi^2 = 185.8$, df = 10, $P < 0.005$). Comparisons within pairs of fish groups (1 with 2, 3 with 4, and 5 with 6) provided evidences of heterogeneity within them (1 with 2: $\chi^2 = 20.09$, $P < 0.005$; 3 with 4: $\chi^2 = 11.4$, $P < 0.005$; 5 with 6: $\chi^2 = 7.17$, $P \approx 0.025$). Therefore, it may be concluded that seasonal effects will be confounded with

fish groups in comparisons between them. It is clear that frequencies of larger fish were higher in later seasons. This may be explained by the fact that as the experiments progressed, fish became larger in the later seasons.

DISTRIBUTION OF GROWTH INCREMENTS

Normal Distribution of Growth Increments

The normal distribution of growth increments of each ration level was tested by Kolmogorov–Smirnov test (Lindgren, 1962). The tables provided 70 tests, all of which showed nonsignificant departures from the normal distribution.

Variance of Growth Increments

The variance of growth increments of each ration level within the six fish groups was estimated as well as degrees of freedom (Table I). Variance estimates give the impression that they increase with ration as well as with the increase of fish weight.

Homogeneity of variance of growth increments within each fish group was tested by Bartlett's test (Table II) as shown by Snedecor and Cochran (1956). The test showed that growth increments had homogeneous variance over all ration levels of 15–24.9-g fish group (excluding increments corresponding to 2.25 g daily ration); over 0.51–5.0-g daily rations of 25–40-g fish group; over 1.51–6.50 g daily rations of 40.1–60-g fish group; over 2.51–7.50-g daily rations of 60.1–90-g fish group; over 1.01–8.0-g daily ration of 90.1–120-g fish group; and over all ration levels of 120.1–211-g fish group (Table I). That is, the minimum of a range of daily rations associated with growth increments having homogeneous variance appeared to increase with the increase of fish weight to 60–90-g fish group and then appeared to decrease with the increase of fish weight. This shifting of ration ranges with increments having homogeneous variance over the groups may be explained by the different ranges of weights of the groups or may be due to changes associated with seasons and confounded with the groups.

The parts of the tables with homogeneous variance will be dealt with in all the following treatments and other parts will be disregarded for simplicity.

This normal distribution of growth increments, homogeneous variance, and more or less fixed ration levels before the start of the experiments satisfy important assumptions necessary to apply the general-linear hypothesis model of full rank with the normal-theory case (Graybill, 1961).

RATION–GROWTH INCREMENT RELATIONSHIP

The ration–growth increment relationship was studied by constructing empirical graphs between the two variables as well as by linear transformation.

Ration–Growth Increment Empirical Graphs

The graphs (Fig. 1) show that growth increments of the 15–24.9-g fish group increased with increasing rations in a concave graph. That is, the rate of increase of growth increments (the slope of the graph) increased

TABLE I. Estimates of variance and degrees of freedom of the fish groups.

Daily ration	Fish groups according to weights (g)											
	15–24.9		25–40		40.1–60		60.1–90		90.1–120		120.1–211	
	Variance	df	Variance	df	Variance	df	Variance	df	Variance	df	Variance	df
0–0.5	1.31833↓[a]	24	0.73221	22	0.41071	7	2.51515	11	2.35285	7	2.89300↓	6
0.51–1.0	1.86666	15	1.90703↓	31	1.62552	99	2.49627	65	3.01664	14	9.43333	9
1.01–1.5	0.33333	2	2.66666	9	1.59064	18	2.74955	33	6.15621↓	38	8.95733	15
1.51–2.0	3.01364	10	2.58810	14	6.34259↓	27	1.80000	4	4.7778	8	10.75000	8
2.01–2.5	0	1	5.20270	11	4.11538	13	–	–	–	–	–	–
2.51–3.0	1.75	3	5.23530	17	6.42857	14	11.24555↓	9	8.33333	3	–	–
3.01–3.5	–	–	7.86670	5	4.25641	12	10.26666	5	3.58333	3	28.00000	2
3.51–4.0	–	–	1.92500	4	6.47620	6	12.02779	8	3.95250	6	–	–
4.01–4.5	–	–	0.50	1	8.00000	4	15.55555	9	9.97062	16		
4.51–5.0	–	–	11.22910	3	6.16666	5	6.49091	10	5.32423	13	12.50000	1
5.01–5.5	–	–	–	–	8.25000	3	16.04444	9	9.35714	7	32.33500	2
5.51–6.0	–	–	–	–	1.33333	2	8.14287	6	17.50000	4	17.00000	3
6.01–6.5	–	–	–	–	12.50000	1	18.00000	1	20.66666	3	18.00000	3
6.51–7.0	–	–	–	–	–	–	12.91666	3	28.00000	2	–	–
7.01–7.5	–	–	–	–	–	–	6.25000	3	40.30000	4	50.00000	1
7.51–8.0	–	–	–	–	–	–	–	–	30.33335	2	0.33500	2
8.01–8.5	–	–	–	–	–	–	–	–	–	–	6.33500	2
8.51–9.0	–	–	–	–	–	–	–	–	–	–	18.45000	4

[a]Arrows show daily rations associated with growth increments having homogeneous variance.

TABLE II. Bartlett's test of homogeneity of variance of each fish group.

Fish group (g)	Range of ration	Chi-square	df	5% level	P
15–24.9	0.0–3.00	4.0206	4	9.49	0.25–0.5
25–40	0.0–5.0	29.007	9	16.919	$P<0.01$
	0.5–5.0	13.860	8	15.507	0.05–0.1
40.1–60	0.0–6.5	49.03	12	21.0	$P<0.001$
	1.51–6.50	3.3417	9	16.9	About 0.95
60.1–90	0.0–7.5	47.304	13	22.4	$P<0.005$
	2.51–7.5	3.051	9	16.9	0.95–0.98
90.1–120	0.0–8.0	30.15	14	23.7	0.001–0.01
	0.51–8.00	26.334	13	22.4	0.01–0.02
	1.01–8.000	20.610	12	21.0	0.05–0.1
120.1–211	0.0–9.0	13.443	12	21.0	0.3–0.5

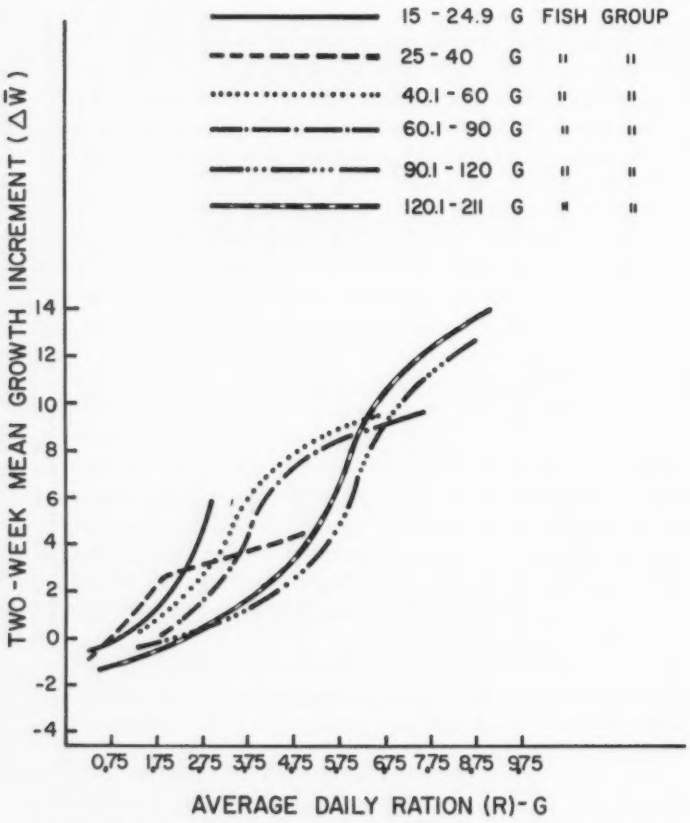

FIG. 1. Daily ration and 2-week growth increments graphs for the six fish groups.

with increasing rations. The graphs of the other groups were sigmoid-shaped without an asymptote. That is, the slope of the graph increased with increasing ration until a maximum slope was attained and then decreased with increasing ration without reaching an asymptote. Therefore, the graph of 15–24.9-g fish group may be considered as the first part of the general sigmoid-shaped graph, that part with increasing slope.

Linear Transformation of Ration–Growth Increment Relation

Many trials were carried out here in an attempt to transform the ration–growth increment graphs from the sigmoid type to the straight line type. The eye test of linearity was considered as an elementary test. When this test gave evidence of a satisfactory criterion of linear graphs for the six fish groups, statistical tests were then applied. The rations (R) and the mean growth increments ($\Delta\bar{w}$) were used for these trials. The transformations which did not yield satisfactory linear plots for all the six fish groups were the following:

$\log(\Delta\bar{w})$	vs.	R
$\Delta\bar{w}$	vs.	$\log(R)$
$\log(\Delta\bar{w})$	vs.	$\log(R)$
$\Delta\bar{w}$	vs.	$\log(\log R)$
$\log(\Delta w)$	vs.	$\log(\log R)$
$\log(\log \Delta\bar{w})$	vs.	$\log(\log R)$
$\log(\Delta\bar{w}/R)$	vs.	R

However, a careful examination of ration–growth increment empirical graphs showed, as explained previously, that the slope decreased if ration was shifted from a certain value that depended on the fish group. Let R_m denote the ration with maximum slope. R_m estimates (Fig. 1) were 2.75, 1.25, 3.25, 3.25, 5.75, and 5.25 for the 15–24.9, 25–40, 40.1–60, 60.1–90, 90.1–120, and 120.1–211 fish groups respectively.

Since the slopes decreased when the rations deviated from R_m values, and if the distances of x-axis on both sides of R_m values are decreased by a certain function, linearity may be attained. The function $\sqrt{|R-R_m|}$, such that it is positive if $R > R_m$ and negative if $R < R_m$, gave satisfactory linear graphs as tested by the eye. Therefore, the zero of the transformed scale is taken at R_m, positive values above it and negative values below it. Let $f(R,R_m)$ denote this function. Plots of other functions of (R,R_m) which were attempted, such as R/R_m vs. $\Delta\bar{w}$, $\log(R/R_m)$ vs. $\log(\Delta\bar{w})$, were unsatisfactory.

Statistical Linearity Test of $f(R,R_m)$ on Δw Relationship

The linearity of the $f(R,R_m)$ on Δw relationship was tested by comparing the mean square of deviations from regression with the mean square within arrays by the Z-test as shown by Fisher (1958) and Lindgren (1962). The total number of degrees of freedom available for each group of fish was the total number of fish with homogeneous variance less 2 df, one of them due to the parameter R_m selected from the data. The Z-test showed nonsignificant departures from linearity within each of the six fish groups (Table III).

TABLE III. Linearity test of $f(R,R_m)$–w relationship.

Fish group (g)	R_m	Mean square: Deviation from regression	df	Mean square: Within arrays	df	Z	5% level
15–24.9	2.75	1.370	4	1.771	54	0.12837	0.86958
25–40	1.25	3.155	7	3.689	94	0.07818	0.59313
40.1–60	3.25	3.669	8	5.900	86	0.23753	0.54577
60.1–90	3.25	18.905	8	10.593	62	0.28984	0.36892
90.1–120	5.75	14.815	13	9.590	108	0.21746	0.29566
120.1–211	5.25	20.303	15	12.244	57	0.25279	0.31739

EMPIRICAL $f(R,R_m)$ ON Δw RELATIONSHIPS

Denoting $f(R,R_m)$ by X and Δw by Y, the linear relationship, $Y = a + bX$, was obtained for the six fish groups in the previous section. The parameters "a" and "b" were estimated by the least-square method (Table IV). The parameter "a" denotes the growth increment when the fish consumes a ration (R) such that $R = R_m$, since in this case $\sqrt{|R-R_m|} = 0$, and $Y = a$.

TABLE IV. Sum of squares and estimates of parameters.

Fish group (g)	Sum of squares: Between arrays	Total	Independent variate	Covariance	a	b
15–24.9	174.8900	270.549	10.634	42.444	6.311	3.991
25–40	255.4406	602.2212	83.5607	139.6386	1.8702	1.671
40.1–60	515.8956	1023.3403	94.5652	214.4465	5.2311	2.2677
60.1–90	494.8445	1151.6713	48.1481	128.6221	4.462	2.6714
90.1–120	1702.3277	2738.0545	137.791	456.100	7.3294	3.3100
120.1–211	2622.9617	3320.8867	176.6875	640.0273	7.3255	3.6223

SIGNIFICANCE OF LINEAR REGRESSION COEFFICIENTS

It has been noted previously that the plaice were fed at three levels, i.e., the rations were more or less fixed before the start of the experiments (Dawes, 1930a, b). Such a condition does not allow a correlation coefficient test. The general linear model of full rank allows tests of significance of regression coefficients from certain constants (Graybill, 1961). The significance of the linear regression coefficients from zero was tested by the t-test as shown by Fisher (1958). The linear regression coefficients of the six fish groups were significantly different from zero at 1% level (Table V).

TABLE V. *t*-Test of significance of regression coefficients from zero.

Fish group (g)	Parameter *b*			Parameter *a*		
	t	df	1% level	*t*	df	1% level
15–24.9	11.12	58	2.575	7.963	58	2.575
25–40	8.60	101	2.575	15.296	101	2.575
40.1–60	11.46	94	2.575	23.467	94	2.575
60.1–90	4.263	70	2.575	13.580	70	2.575
90.1–120	10.094	121	2.575	9.74	121	2.575
120.1–211	10.68	72	2.575	8.046	72	2.575

HOMOGENEITY OF MEAN SQUARE OF DEVIATION FROM LINEAR REGRESSION

It is important to test homogeneity of mean square of deviation from linear regression before testing the hypothesis of significant differences between parameters of the linear relationships between fish groups. Heterogeneity of mean square of deviation from linear regression is evidence of different linear relationships. Bartlett's test as well as variance ratio test (F) were used for this purpose (Snedecor and Cochran, 1956). Barlett's test gave evidence of heterogeneity of the variance among the six fish groups ($\chi^2 = 10.032$, df = 5, P lies between 0.01 and 0.001). Eliminating the 15–24.9-g fish group, the test gave evidence of heterogeneous variance among the remaining five groups ($\chi^2 = 10.894$, df = 4, $0.05 > P > 0.02$). Eliminating the 15–24.9- and 25–40-g fish groups, evidence of homogeneous variance was obtained for the remaining four groups ($\chi^2 = 5.906$, df = 3, $0.2 > P > 0.1$). The 15–24.9- and 25–40-g fish groups gave evidence of homogeneous variance ($F = 2.302$, $n_1 = 7$, $n_2 = 4$, 5% level = 6.09).

COMPARISON BETWEEN VARIOUS $f(R,R_m)$ ON Δw RELATIONSHIPS

Analysis of covariance was used to test for significance of differences between parameters of $f(R,R_m)$ on Δw relationships of the six fish groups as shown by Snedecor and Cochran (1956).

The slopes (b) of the 15–24.9- and 25–40-g fish groups were found significantly different ($F = 20.265$, $n_1 = 1$, $n_2 = 11$, 5% level = 4.84). That is, fish belonging to the first group gained weight at a higher rate than those of the second group. The slopes (b) of the other four fish groups were not significantly different from each other ($F = 2.76$, $n_1 = 3$, $n_2 = 44$, 5% level = 2.82). The mean levels of growth increments (a) were, however, found to be significantly different ($F = 7.069$, $n_1 = 3$, $n_2 = 47$, 5% level = 2.805). The 90.1–120- and 120.1–211-g fish groups gave evidence of homogeneous parameters (b: $F = 2.354$, $n_1 = 28$, $n_2 = 1$, 5% level = 249.6; a: $F = 3.46$, $n_1 = 29$, $n_2 = 1$, 5% level = 249.8).

Therefore, there is evidence of significant differences between ration–growth increment relationships of 15–24.9- and 25–40-g fish groups as well as relationships of the other four groups. However, there is evidence of identical relationships of 90.1–120- and 120.1–211-g fish groups (Table VI). These significant differences between fish groups may be true differences associated with different weight ranges of the groups, may be due to seasonal effects confounded with the groups, or due to both factors. Paloheimo and Dickie (1966b) suggested that body weight is not the determining factor as long as environmental conditions are within the normal biokinetic range.

TABLE VI. Analysis of covariance of the various fish groups.

Fish group (g)	df	Σx^2	Σxy	$\Sigma\left\{n_p(\bar{y}_p-\bar{y})^2\right\}$	Deviation from regression		
					df	Sum of squares	Mean square
15–24.9	5	10.6340	42.444	174.8900	4	5.4810	1.370
25–40	8	83.5607	139.6386	255.4406	7	22.0901	3.155
Within					11	27.5711	2.506
Regression coefficient					1	50.7857	50.7857
Common	13	94.1947	182.0826	430.3306	12	78.3568	6.5297
					1	63.1714	63.1714
	14	212.933	281.431	513.4921	13	141.5282	
90.1–120	14	137.691	456.100	1702.328	13	192.600	14.815
120.1–211	16	176.687	640.027	2622.962	15	304.549	20.303
Within					28	497.149	17.755
					1	7.542	7.542
Common	30	314.478	1096.127	4325.290	29	504.691	17.403
					1	5.029	5.029
	31	319.587	1108.800	4356.670	30	509.720	
40.1–60	9	94.565	214.446	515.895	8	29.593	3.699
60.1–90	9	48.148	128.622	494.844	8	151.245	18.905
90.1–120	14	137.791	456.100	1702.327	13	192.600	14.815
120.1–211	16	176.687	640.027	2622.962	15	304.549	20.303
Within					44	677.987	15.409
					3	127.589	42.529
Common	48	457.191	1439.195	5336.028	47	805.576	17.139
					3	363.472	121.157
	51	678.213	1796.263	5926.492	50	1169.048	

DISCUSSION

Some implications of the empirical $f(R,R_m)$ on Δw relationships are discussed here.

Maintenance Diets

Maintenance diets, i.e., diets associated with no change of weights, estimated by means of the equations were 0.25, 0.87, and 1.17 for 15–24.9-, 90.1–120-, and 120.1–211-g fish groups respectively. Estimates of the other groups were eliminated because their maintenance rations fell in the ranges with heterogeneous variance relative to the rations used for the derivation of the equations. Extrapolations in these cases are incorrect. In fact, the maintenance ration of the 90.1–120-g fish group lay on the boarder-line between the data with different variance but may be considered here.

Considering the central weight of each group as the representative weight, the maintenance ratios were estimated at 0.25/20, 0.87/105, and 1.17/165 with the following conclusions: (1) the maintenance ration may increase with increasing fish weights; (2) the maintenance ration per unit weight estimated at 0.0125, 0.0083, and 0.0071 respectively, probably decreased with increasing weight. (Confounding with temperature affects these estimates and casts doubt on the above two conclusions. Dawes (1930a, b) supported these two conclusions.) (3) log (maintenance ration) = −0.602, −0.061, 0.068; log (mean weight) = 1.301, 2.021, 2.217. The points of the above two variables lie on a more or less perfect straight line with a slope of log (maintenance ration) on log (mean weight), γ, estimated at 0.73. The slope, γ, is known as the "weight exponent" which is a parameter in the relation, $T = \alpha W^{\gamma}$, where T is total metabolism and W is weight. Winberg (1956, 1961) concluded that at levels of metabolism close to the "standard" or "nonactive" the exponent, γ, characterizes almost all fish species and takes a value close to 0.8. Paloheimo and Dickie (1966a) used Dawes' (1930a, b) data and obtained estimates of $\gamma = 0.63 \pm 0.06$, 0.62 ± 0.06, and 0.96 ± 0.2 for maintenance, ad libitum, and intermediate feeding levels respectively and after correcting to 20 C. They explained this low estimate for the first two levels of feeding by the interactions of metabolism–body-size relationship with temperature and the design of the experiments. Weights of the four intermediate rationed fish ranged from about 30 to about 60 g except four weights which ranged from 20.3 to 26 g, with three records of growth increments. Therefore, the data supplied by intermediate rationed fish were more or less not included in the three tables which provided the "γ" estimate at 0.73. That is, the data supplied by the intermediate rationed fish cannot be considered as the reason of the higher, γ, estimate than these by Paloheimo and Dickie (1966a). Seasonal effects confounded with the groups may account for the difference between the two estimates. Another estimate of γ at the level of feeding with maximum growth efficiency (the level of expected maximum efficiency shown in the next section) was 0.577. This estimate was obtained by using 0.8 as a constant correction term of rations consumed to rations assimilated (p coefficient) for all fish groups. The deviation of the latter estimate of γ from the accepted value (0.8) may be due to confounding of seasonal effects with fish groups, incorrectness of the hypothesis of constant p value for the six fish groups, the

probable variation of p value through the different seasons, or a combination of these factors. Johnson (1966) did not find apparent decrease of maintenance ration-per-unit weight with increasing weight in pike.

Growth Efficiency

The ration–growth increment relationships provide estimates of expected growth increments (E Δw) at each ration level (R). Expected efficiencies of growth (E $\Delta w/R$) were calculated. Expected maximum efficiency of each fish group occurred at R_m for 15–24.9-g group; $R_m + 0.5$ for 25–40-, 40.1–60-, and 60.1–90-g groups; and $R_m + 1$ for 90.1–120- and 120.1–211-g fish groups. That is, the expected maximum efficiency occurred at $R \approx R_m$ which lay between maintenance and maximum rations for all groups except the smallest (15–24.9 g) group. Dawes (1930b) concluded that male and female plaice alike made relatively much more efficient use of food for purposes of growth when supplied with certain intermediate rations between their maintenance requirements and maximum demands. Brody and Proctor (1943) and Kostitzin (1939) considered that more efficient utilization of food takes place at a ration level below the maximum.

The plots of logarithms of expected growth efficiency against ration gave rise to approximately similar graphs for the six fish groups (Fig. 2). The graphs are characterized by an irregular ascending limb to R_m approximately and

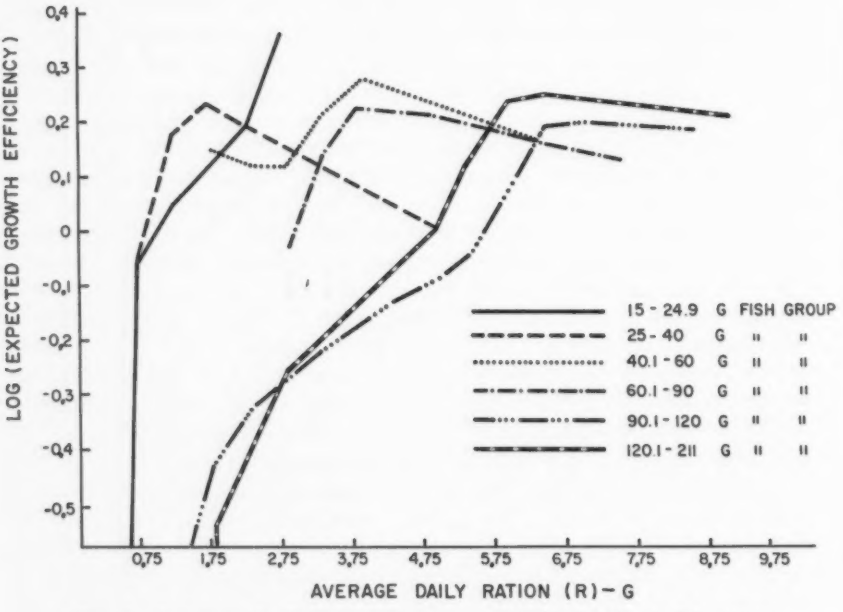

FIG. 2. Log (growth efficiency) and ration relationship for the six fish groups.

then take the form of a descending straight line with increasing rations. The 15–24.9-g fish group showed only the ascending part because rations did not surpass R_m. Paloheimo and Dickie (1966b) considered that the logarithms of growth efficiency (log K) decreases linearly with increase in rations when fish are fed on one type of food, i.e., $\log (\Delta w/R\ \Delta t) = -a-bR$ and $\Delta w/\Delta t = R\ e^{-a-bR}$. Re-examination of Paloheimo and Dickie's (1966b) analysis of experiments which showed negative significant correlation between log(K) vs. R, viz. Dawes (1930a, b) plaice experiments, Pentelow's (1939) brown trout, Hatanaka et al.'s (1956a, b) plaice experiments, Menzel's (1960) reef-fish experiments, Gerking's (1952) bluegill sunfish experiments, and Kinne's (1960, 1962) desert pupfish experiments, showed that in all cases the experiments were carried with fish fed ad libitum all day or for sometime during the day. That is, the fish probably had occasion to obtain rations that surpassed R_m with the result of a linear decrease of log(K) with increasing rations. In other experiments the level of feeding was not mentioned and some experiments showed significant negative correlation whereas others failed to show it. The high scatter which characterized Brown (1946) data for brown trout fed ad libitum and restricted ration may be explained that fish fed ad libitum may consume less than R_m or a change of R_m value during the experiment. As log(K) increases with increasing ration from the maintenance ration to a maximum and then decreases linearly with increasing rations, as shown in Fig. 2, the relation $\log(K) = -a-bR$ may be a special case, and not a general one. This may explain the recorded insignificant correlation coefficients and the small value of some significant correlations in many experiments discussed by Paloheimo and Dickie (1966b). Confounding with weight is probably another source of these decreased estimates of correlation coefficients.

A study of the linearly descending parts of R/log(K) graphs of the five fish groups may be as follows (where "*a*" and "*b*" are the straight line parameters):

Fish group	*a*	*b*	df	Correlation coefficients	1% level
2	0.3608	−0.07332	5	−0.996	0.874
3	0.4059	−0.03811	4	−0.997	0.917
4	0.3473	−0.02952	6	−0.993	0.834
5	0.2683	−0.01024	2	−0.966	0.990
6	0.3402	−0.01493	4	−0.984	0.917

Therefore, it is clear that there was a very high correlation coefficient between R and $\log(\Delta w/R\Delta t)$ within the groups when R surpassed R_m. Analysis of covariance was used to test the hypothesis that the slopes of the five groups do not deviate significantly from each other (Table VII). Assuming homogeneous mean square of deviation from regression for the five groups, the regression coefficients differ significantly from each other ($F = 570.2$, $n_1 = 1$,

TABLE VII. Analysis of covariance of the linear descending part of the relationship R on log $(\Delta w/R\Delta t)$.

Fish group	df	Σx^2	Σxy	Σy^2	Regression coefficient	Deviation from regression: df	Deviation from regression: $\left[y^2-\frac{(\Sigma xy)^2}{\Sigma x^2}\right]$	Deviation from regression: Mean square
2	6	7.000	−0.51330	0.03729	−0.07332	5	0.000288	0.0000576
3	5	4.375	−0.16675	0.006381	−0.03811	4	0.000026	0.0000065
4	7	10.500	−0.30995	0.009264	−0.02952	6	0.000115	0.0000175
5	3	1.250	−0.01280	0.000140	−0.01024	2	0.000009	0.0000045
6	5	4.375	−0.06530	0.001006	−0.01493	4	0.000032	0.000008
Within						21	0.000470	0.00002238
Regression coefficient						1	0.012763	0.012763
Common	26	27.5	−1.0681	0.054718	−0.03884	20	0.013233	0.00066165

n_2 = 21, 5% level 4.32). The difference between the slopes may be explained by confounding of seasonal effects with fish groups, due to real differences between ration–growth increment relationships of the different weight ranges of groups, or due to a combination of the two factors. The significant variations between the straight lines of R/log(K) graphs of the groups may show the probable error confounded with weight variations within certain limits. Johnson (1966) did not observe a consistent trend in growth efficiencies of pike as rations diminished.

FISH WEIGHT AS A GROWTH-DETERMINING FACTOR

An important consequence of the $f(R,R_m)$ on Δw relationship is that fish growth depends on the parameter R_m. Significant differences between these relationships in the different groups of fish weights were attributed to true differences due to different weight ranges, i.e., fish weights within certain limits are determining factors; confounding seasonal effects with the fish groups; or a combination of the two factors. The nonsignificant differences between ration–growth relationships of 120.1–150- and 150.1–211-g fish groups, although their distributions within the seasons were significantly different, shows that fish weights as a growth-determining factor may be more important than the seasonal effects within 120.1–211-g range of the fish weight and seasons of the experiments. The probable dependence of ration–growth increment relationship on fish weight indicates that growth may be the result of interaction between ecological factors, offering food and other environmental factors, and physiological factors determined by fish weight. This was concluded by many investigators and favours the probability that differences between $f(R,R_m)$ on Δw relationships were probably partly due to different weight ranges of the fish groups. Brody (1945) visualized growth of fish as

a series of growth stanzas which are entered by ecological and physiological size thresholds, and within which, size is the basic determinant of both ecological and physiological opportunity for growth. Brown (1946), Stringer and Hoar (1955), Newman (1956), and Kalleberg (1958) describe growth rates influenced by size hierarchies in groups of fish. Larkin et al. (1957) suggest that size of rainbow trout (*Salmo gairdneri*) is a better indicator of growth opportunity than age. They also suggest new growth opportunities when the individuals cross size "thresholds." Martin (1949) related sharp breaks in relative growth lines for several species of fish to ossification and maturity. Hiatt (1947) showed that *Chanos chanos* of approximately 100 mm in body length undergoes a sharp break in relative growth of gut length. Parker and Larkin (1959) regarded growth in fishes as a series of stanzas within each of which the growth rate is proportional to a constant multiplied by a fractional power of weight. It seems, therefore, that there is a considerable body of information on fish growth in nature which suggests that they may frequently encounter situations in which the ration obtained may be less than R_m, that required by their physiological condition (weight relation) to support maximum growth efficiency.

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Condition of Newfoundland Trap-Caught Cod and Its Influence on Quality After Single and Double Freezing[1,2]

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ABSTRACT

Quality assessments, based on organoleptic evaluations and chemical tests, were conducted before and after cold storage, on trap-caught cod (*Gadus morhua*) which was unfrozen, once-frozen, or twice-frozen. The cod was processed both before and during rigor, after icing or after handling without ice, at various times during the catching season.

Lactate determinations and *p*H measurements on whole fish at killing showed a seasonal variation. There was a correlation between lactate determinations and *p*H measurements in freshly killed and freshly frozen fillets that had been frozen both before and during rigor. Icing immediately after killing slowed the rate of lactate accumulation.

Differences in the state of pre- and in-rigor chilled fish at freezing, as assessed by muscle glycogen, lactate, and reserves of ATP, resulted in differences in texture, thaw-drip, and *p*H. Patterns in texture may be predicted by the amounts of thaw-drip and the *p*H measured in the cold-stored pack.

Intensity of mealiness and short-grained features in freshly frozen (once) fish was correlated with the level of ATP immediately before cooking. Fillets frozen before rigor toughened very slowly in storage at –23 C and the mealy condition eventually disappeared. Hence, fish downgraded for mealiness at freezing improved or maintained initial overall texture scores during 6 months in storage. Although the panel showed a clear preference for fish frozen pre-rigor, it would be impossible for industry to freeze more than a portion of the landings pre-rigor. To obtain the next best once-frozen product, chilled in-rigor fish should be processed.

Double freezing gave the next best product from chilled, prerigor fish. Fish frozen pre-rigor in the "dressed" condition and stored at –23 C, then thawed, processed, and refrozen at 16 weeks, was equal in quality to once-frozen controls. The texture in the twice-frozen material prepared from iced, prerigor frozen fish dropped after a further 10 weeks storage, making it little better than the twice-frozen product of commercial-like handling. A very satisfactory twice-frozen product was obtained also by holding unfilleted blocks of non-iced, gutted fish at –46 C

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[2]This paper, part of which contains material discussed at the FAO Technical Conference (held in Madrid, September 4–8, 1967) is the thirteenth in a series on quality of Newfoundland fish. Earlier papers are listed by MacCallum et al. (1966, 1967a, b).

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before thawing and refreezing, followed by remaining storage at −23 C. However, such low temperature storage is not within the scope of present commercial operations.

With pre- and in-rigor frozen material a correlation was found between thaw-drip and *p*H, thaw-drip and overall texture (in-rigor freezing only), *p*H and overall texture values, and between *p*H and toughness. There was also a correlation between extractable protein nitrogen (EPN) values and overall texture scores of fish frozen in various stages of rigor (encountered during unfrozen storage of upwards of 24 hr before processing) but not between EPN and overall acceptability. With twice-frozen fillets prepared from material also handled unfrozen as above, there was significant correlation between EPN values at tasting and overall texture scores and between EPN and overall acceptability scores.

Free fatty acid (FFA) values of the trap fish were high and showed no increase as the result of thawing and refreezing or further storage at −23 C or with pre- and in-rigor freezing. Storage at −46 C resulted in less FFA development. There was significant correlation between EPN and FFA values for June-caught once-frozen fish and between FFA values and strictly taste scores for June and July fish but no correlation between FFA and overall texture scores, or in the case of twice-frozen fish of any particular pre-freezing treatment, between FFA and taste, overall texture, or overall acceptability.

INTRODUCTION

STUDIES on the effect of handling and freezing trap-caught cod (*Gadus morhua*) have shown that quality was dependent on the physiological condition of the muscle at capture, which in turn was related to feed and environmental conditions (Idler et al., 1964; MacCallum et al., 1966, 1967b). Better cold-storage quality was obtained in in-rigor frozen fish caught at the beginning and end of the trap fishery than in those taken at the height of the run in July (MacCallum et al., 1966, 1967b). It was found that if rigor had set in, the catch should be iced or chilled in the whole, gutted form for about 24 hr while awaiting processing (MacCallum et al., 1967b). Headed, gutted, in-rigor iced fish, twice-frozen (second freezing after thawing in tap water and filleting) using fish obtained throughout the catching season of May–August produced a marketable product (Dyer et al., 1962 (July fish only); MacCallum et al., 1964b, 1966). Polyphosphate treatment of fillets of trap fish, either before single freezing or before the second freezing of a double-freezing operation, was found to be beneficial, promoting maximum yields on packing, reducing thaw-drip (MacÇallum et al., 1964a, b), and improving textural characteristics in the once-frozen pack (MacCallum et al., 1964a).

The present paper reports additional studies, in particular those dealing with the enhanced cold storage qualities (as indicated by taste-panel assessment of overall texture) of both once- and twice-frozen cod frozen before rigor compared with fish allowed to proceed into rigor in ice under controlled or under commercial-like conditions. The authors also examine the quality of pre-rigor frozen material prepared from fish taken at different times during the trap season. Previous work on unfrozen fillets (MacCallum et al., 1967b) showed the correlation of free drip with *p*H and a direct association of free drip and state of feeding as indicated by muscle glycogen at killing. The level of glycogen at killing was shown to vary with the season of catch. Low glycogen values were reported before the fish had become glutted on capelin (*Mallotus*

villosus) and after feeding had tapered off whereas high values were obtained in the intervening weeks (mid-July to mid-August) when the fish were feeding heavily. The surface seawater temperatures were 7–12 C in this mid-July to mid-August period compared with 3–5 C in mid-June. Fish initially high in glycogen were shown to drip excessively if filleted before rigor and held in chill storage and to drop to a low *p*H, sometimes as low as 6.1–6.2. The poorest texture after single freezing and cold storage was generally associated with fish which, if alternatively stored as chilled fillets, would drip excessively and drop to a low *p*H. The present work explores this area further with attention to the interrelationships of: *p*H, texture, and thaw-drip; FFA, EPN, and taste-panel scores (i.e. taste, overall texture, and overall acceptability). The textural quality expressed as mealiness (Idler et al., 1964), its correlation with the concentration of adenosine-5-triphosphate (ATP) in the frozen fillet, and its effect on frozen storage quality was studied. The following relationships were also investigated: glycogen/lactate and temperature/rate of lactate accumulation (unfrozen samples); *p*H/toughness (frozen samples) and state of rigor at freezing/frozen storage quality. In addition, the lactate/*p*H relationship was established for both unfrozen and frozen samples.

EXPERIMENTAL

Handling

One portion of each of three catches of fish (51–61 cm overall length) handled commercially (without ice) in 1962 was eviscerated between time of capture and landing 3 hr later. On June 29, July 19, and July 31 processing, packing into 1-lb cartons, overwrapping in wax paper, and plate freezing (0 to −15 C in less than 1 hr, the fish having been removed from the freezer after reaching a temperature of about −32 C) were completed within 5½ hr of capture. On each date a second portion of eviscerated fish from the same catches, kept uniced in the shade for an additional period before filleting, was processed and frozen within 10½ hr of capture. Fish from other hauls taken at the same times were gutted and iced at the trap, held 24 hr, filleted, and frozen as controls. The temperatures of uniced fish at filleting and freezing were 10 C (June 29), 9–11 C (July 19), and 13–15 C (July 31). All packages were cold stored at −23 C and assessed by the taste panel.

In the 1963 and 1964 trap-fishing seasons cod were stunned, bled, eviscerated, and iced at the traps on several occasions commencing June 19 and ending August 10 and filleted before and during rigor (24 hr iced storage), then frozen and stored and assessed for texture and thaw-drip values.

On June 21, 1965 fish were taken from a trap, stunned, bled, sampled for muscle glycogen (analyses reported by MacCallum et al., 1967b) gutted, and iced at sea and later filleted and frozen, half before and half during rigor, and were held in frozen storage at −23 C and taste-tested.

Catches on June 29 and on July 27, 1966 were divided after gutting at sea, one lot iced immediately, another left uniced. From the iced lot 60 lb of

dressed fish were frozen in a 4-inch-thick block within 3 hr of capture. Freezing was accomplished in a commercial vertical plate freezer with the fish in the centre of the block being lowered from 0 to −15 C in less than 3 hr. The block was wrapped in 4-mil (0.004-inch) polyethylene sheet and sealed with masking tape. For controls, the rest of the fish from the iced lot were filleted, skinned, and frozen within 1 hr in 1-lb packages. The uniced lot was divided 9 hr post-mortem. From the first half, 60 lb of dressed cod were immediately frozen in a 4-inch-thick block and at the same time other fish were filleted, skinned, packed, and frozen in 1-lb packages in 1 hr. The other half was iced for an additional 15 hr after which time 60 lb of dressed fish were frozen in a 4-inch-thick block and the remainder processed and frozen in 1-lb packages. Four months after freezing, all blocks were defrosted in recirculating tap water at 18 C and the fish processed using the same methods for packing and freezing used with once-frozen fish. All fillets were packed in 1-lb waxed paper cartons and overwrapped with Saran after freezing.

Packages contained fillets from more than one fish except for those prepared in 1965 and from June 28, July 28, and August 16, 1966 catches, which were packed with fillets of one fish only.

Cold storage temperature for all fish was −23 C unless otherwise noted in the text.

Laboratory Procedures

The *p*H measurements of unfrozen fish were made by inserting the tip of a combination glass/calomel electrode into a fresh incision below the first dorsal fin in whole, eviscerated fish or into the cut surface of a fillet at a point located about one-third the length of the fillet measuring from the head end. To obtain a value for frozen fish, a sample of approximately 20 g was cut from the frozen package, placed in a polyethylene bag and immersed in cool water until substantially thawed.

Free fatty acids were determined on frozen fillets of fish caught June 29 and July 27, 1966 using a 50-g sample extracted with a chloroform–methanol mixture. The chloroform phase was allowed to separate from the methanol phase, and covered to avoid evaporation. The methanol layer was aspirated leaving the chloroform layer for determinations of FFA and total lipids. Two aliquots were used for the measurement of FFA by titration with alcoholic NaOH and one aliquot used to determine total lipids (T.L.) by the method of Bligh and Dyer (1959).

The methods used for estimating thaw- and free drip respectively were described by MacCallum et al. (1966, 1967b). Thaw-drip was collected in a vessel from a weighed sample of approximately 50 g taken from a frozen package. The sample was laid on a mesh screen which, in turn, was supported above the fluid, the whole being enclosed in airtight polyethylene to prevent evaporation and stored at 4 C. Duplicate samples were weighed at the start and at 48 hr after storage, the results averaged and used in calculating the percentage thaw-drip. This was done on fish caught on the following dates: June

19, July 8, July 14, and August 10, 1964; and June 29 and July 27, 1966. Free drip was collected from a single, whole, unfrozen fillet in an identical manner to thaw-drip. The fillet was weighed at the start and at 24 hr after storage at 4 C and the results used in calculating percentage free drip. This was done on fish caught on June 19, June 27, July 28, and August 10, 1964; June 15 and August 23, 1965; and June 29 and July 27, 1966.

At killing on June 28 and July 28, 1966, samples for adenosine 5′-triphosphate (ATP) and lactate determinations were obtained posterior to the first dorsal fin above the lateral line, and samples for glycogen determinations were taken adjacent to the ATP-lactate samples on the same side of the fish. Before filleting and freezing, another set of ATP, lactate, and glycogen samples were taken from the same position but on the opposite side of the fish. ATP and lactate determinations were also made on samples from stored frozen fillets of fish caught on June 28 and July 28, 1966 and lactates only on fish caught June 29, July 27, and August 16, 1966.

For determining ATP and lactate concentrations, a sample of approximately 10 g from both the unfrozen fish and frozen fillets was immediately placed in liquid nitrogen after removal from the fish or package. Later, an extract was prepared in a refrigerated room (2 5 C) from the frozen sample. The latter was powdered in a porcelain mortar with frequent additions of liquid nitrogen to prevent thawing. A measured amount of 6% perchloric acid was added such that a ratio of 4:1 of total fluid volume to the weight of the muscle was achieved. The mixture was blended, centrifuged, and divided into two aliquots which were titrated with 5 M potassium carbonate using methyl orange as an indicator (Lamprecht and Trautschold, 1963).

Duplicate aliquots of each of the above extracts were used for ATP determinations. Prepackaged reagents contained in Calsuls (Calbiochem) were used. Glucose was phosphorylated in the 6 position with hexokinase. Glucose-6-phosphate so formed was then oxidized by glucose-6-phosphate dehydrogenase in the presence of triphosphopyridine nucleotide (TPN). An amount of reduced triphosphopyridine nucleotide (TPNH) proportional to the concentration of ATP in the sample was produced and measured spectrophotometrically at 340 mμ.

Duplicate aliquots of the above-mentioned titrated extracts were used for the lactate estimation of both unfrozen and frozen samples. The method was essentially that of Hohorst (1963) modified slightly to permit the use of more convenient volumes. The extract was further diluted with distilled water, buffered with hydrazine-glycine and diphosphopyridine nucleotide (DPN) and lactic dehydrogenase (LDH) added. The concentration of lactate was determined by following the increase of optical density spectrophotometrically at 340 mμ.

For determining muscle glycogen in all whole fish, about 5 g of muscle, excised as described above, were immediately placed in tubes containing 10 ml of 30% KOH. These tubes were held chilled in ice on the vessel and at 4 C ashore until the digestion procedure could be undertaken. The muscle,

of known weight, in the tubes was digested in a water bath at 100 C for 1 hr. After cooling, the digests were made up to 25 ml with 30% KOH and two 5-ml aliquots taken. Glycogen was precipitated with 96% ethanol, according to Carroll et al. (1956), redissolved with distilled water, and estimated colorimetrically using the phenol–sulphuric acid reagent of Montgomery (1957). Fish on which glycogen determinations were made were caught on June 21 and July 19, 1965, and June 28, July 28, and August 16, 1966.

Frozen samples from fish caught on June 29 and July 27, 1966 were analyzed for extractable protein nitrogen. Values for 1 and 16 weeks frozen storage were determined using two separate 22.5-g samples from each of two 1-lb packages. However, after 26 weeks frozen storage, a single 22.5-g sample from each of the two 1-lb packages was extracted. The samples were blended for 2.5 min with a buffer consisting of 0.6 M NaCl and 0.003 M $NaHCO_3$ in a Waring Blendor at a speed of 7000 rpm. The extracts were centrifuged for 30 min at 2000 rpm (Dyer et al., 1950, 1964) in a cold room at 2–5 C. Colorimetric readings were made after the addition of biuret solution following the procedure of Snow (1950).

Taste panels were conducted as described by MacCallum et al. (1966). The score for overall texture includes an assessment for mealiness, toughness, and other textural characteristics. Rubberiness, stringiness, extreme chewiness, and toughness are the most common qualities attributed to cod in scoring unacceptable or lower texture grades. The score for mealiness is the texture value which would have been given the sample had this been the only characteristic assessed. A similar approach was followed in assessing toughness alone. In calculating the overall acceptability score for cod, the authors equated it with the overall texture score except where a difference of more than 20 points arose between overall texture and taste scores. When the difference was greater than 20 the overall texture and taste scores were averaged to give the overall acceptability score. The fish tasted were caught on June 27 and July 19, 1962; June 19, July 8, July 14, and August 10, 1964; June 4, June 21, July 19, and August 2, 1965; and June 28, June 29, July 27, and July 28, 1966.

Statistical analysis of the data was carried out according to the formulae described by Snedecor (1956).

RESULTS AND DISCUSSION

Glycogen, Lactate, and *p*H Before and After Freezing

Other workers have studied the concomitant aspects of glycogen depletion and lactate accumulation but a stoichiometric proportion has not always been found (Partmann, 1965). The present work shows a balance between average amounts of glycogen and lactate at killing and again after 2 weeks frozen storage, although changes in glycogen and in lactate for the individual fish did not correlate significantly. The samples averaged 0.11% lactate at the June 28, 1966 killing, and 0.20% on July 28 (Table I). The *p*H of the June

TABLE I. Changes in muscle glycogen, lactate, and *p*H at killing and at tasting (0–2 weeks frozen storage) in iced trap fish frozen before and after rigor in 1966.

Date of catch	No. of fish	Hr post-mortem filleted & frozen	State of rigor at freezing	At killing			At tasting (after 0–2 weeks frozen storage)		
				*p*H	Glycogen (*mg/100 g*)	Lactate (*mg/100 g*)	*p*H	Glycogen (*mg/100 g*)	Lactate (*mg/100 g*)
June 28	15	5	Pre-rigor	7.25 ±0.04	349 ±62.5	113 ±9.1	6.42 ±0.06	175 ±50.0	300 ±17.1
	5	29	In-rigor	7.31 ±0.05	307 ±63.9	94 ±17.3	6.40 ±0.09	55 ±24.8	345 ±17.8
July 28	15	5	Pre-rigor	7.04 ±0.04	–	209 ±12.5	6.48 ±0.04	141 ±29.1	379 ±26.1
	5	10	Incipient to full	7.07 ±0.13	–	179 ±12.1	6.61 ±0.09	61 ±11.5	366 ±22.7

fish at killing was higher than that of the July fish. The muscle glycogen in the June 28 prerigor frozen samples dropped 174 mg/100 g between killing and post-freezing analysis, whereas the lactate increased 187 mg/100 g during the same period. Respective differences for in-rigor samples of the same catch were 252 and 251 mg/100 g.

In the two catches referred to above, significant (1%) correlation was found between lactate and *p*H values. Figures 1 and 2 show the lactate and *p*H values at killing and after freezing with additional points for the July 28 samples at filleting.

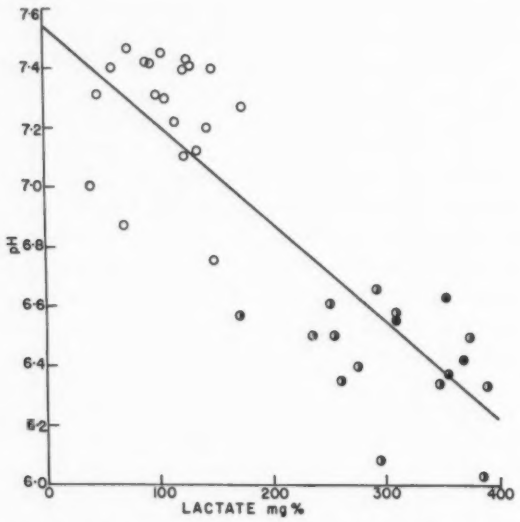

FIG. 1. Correlation of *p*H and lactate in fish of the June 28, 1966 catch using measurements taken on the same individual fish at killing and after freezing fillets of iced fish before or during rigor. At killing ○; prerigor frozen samples ◑; in-rigor frozen samples ●. $r = 0.81$, df = 35.

With trap fish, immediate icing after killing slowed the rate of lactate accumulation (Fig. 3) as shown from examination of 12 fish stunned and killed at the traps on August 16, 1966, 6 being iced, 6 uniced. From a level of 0.18% ± 0.011 ($n = 12$) at killing, the lactate content of the uniced fish had increased 100% after 9 hr storage, that of the iced fish by 33%. At this time average *p*H of the iced fish was 6.85 compared with 6.67 for the uniced. There was a correlation (1% significance) between the muscle lactate of individual fish and the *p*H at the same time, in agreement with results obtained from the June and July fish (Fig. 1 and 2).

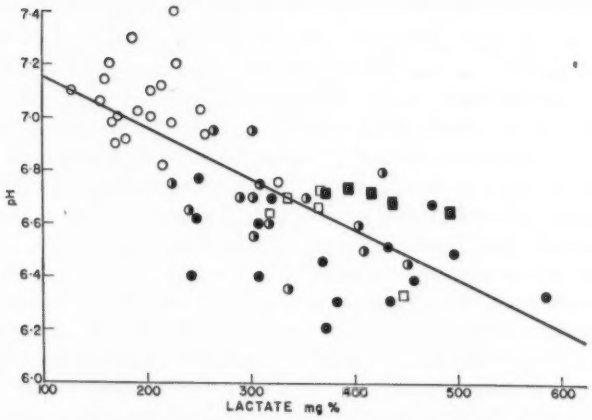

FIG. 2. Correlation of *p*H and lactate in fish of the July 28, 1966 catch using measurements taken on the same individual fish at killing, at filleting iced fish 5 and 10 hr post-mortem, and after freezing. At killing ○; at filleting: 5 hr post-mortem ◑, 10 hr post-mortem ■; after freezing: 5 hr post-mortem ●, 10 hr post-mortem □. $r = 0.73$, df = 58.

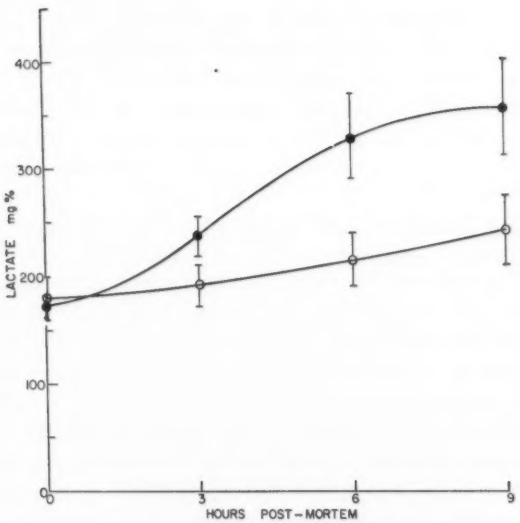

FIG. 3. The rate of lactate accumulation in iced (open circles) and non-iced (solid circles) eviscerated trap-caught cod taken on August 16, 1966. Each point represents the average of one lactate determination on each of six fish. The standard error of each average is also shown.

Mealiness and Toughness in Relation to Pre- and In-Rigor Freezing

Fillets of iced trap cod taken early in the season and single-frozen before rigor usually produce an excellent product for cold storage. Yet these and later caught fish, immediately upon freezing, can have a granular or "mealy" texture (Idler et al., 1964) which results in some downgrading. Jones (1965) notes that this texture is prized in some Scandinavian markets where it is accepted as a token of freshness at processing. Borgstrom (1961) also comments on the emphasis placed by the Russians on prerigor freezing so as to prevent any detrimental pre-freezing changes in the tissue structure. Mealiness is attributed to contraction occurring during cooking and tasting. Short-grained features, possibly a milder form of the mealy phenomenon, are also associated by the panel with fresh-frozen fish, even after cold storage up to 3–6 months. We find that the degree of intensity of mealiness and short-grained features (the former characteristic being dominant) in freshly frozen trap fish can be correlated with the level of ATP in the frozen fish prior to cooking. Analysis of two catches (June 28, July 28, 1966) prepared as described earlier, showed this relation, based on scores for individual fish, to be very significant (Fig. 4).

Results obtained in 1964 and 1965 showed the mealy characteristics to disappear almost completely in 3–4 months from iced-at-sea fish, frozen before rigor and cold stored (Fig. 5). Toughness, noted to a slight extent in these fish initially, did not increase at all in the 1964 fish during cold storage and increased only a moderate amount in the 1965 cold-stored samples. The disappearance of the mealy characteristic thus removed one of the major features tending to cause an initial lowering in overall texture rating. Hence, fish that were mealy after freezing actually improved or at least maintained initial overall texture score during the first 6 months storage. This result is not commonly encountered in the preservation of fish by freezing (MacCallum et al., 1966; Peters et al., 1968).

Shortly after freezing a taste panel gave both the prerigor and in-rigor fillets (June 21, 1965), prepared as described earlier, an average overall texture rating of 70 (n = 311) on a 0–100 scale (Fig. 6). In the prerigor frozen fillets mealiness and short-grained characteristics rather than chewiness were the main reasons for downgrading, whereas in the in-rigor frozen fish the score was a true reflection of the toughness. Regression of overall texture score on time during 12 months frozen storage (Fig. 6) illustrate the clear preference shown by the panel for the prerigor frozen material, and the substantial and unique benefits made possible by cold storing these cod in contrast to similarly storing fish which have been frozen during rigor.

Fairly low initial overall texture scores at 61.8 (prerigor frozen), and 55.6 (in-rigor frozen) for the June 28, 1966 fish, and 55.3 (prerigor frozen) for the July 28, 1966 fish (Table II) indicated that these two catches, discussed in the section immediately preceeding, contained material which yielded a frozen pack typical of the poorest production (as defined by MacCallum

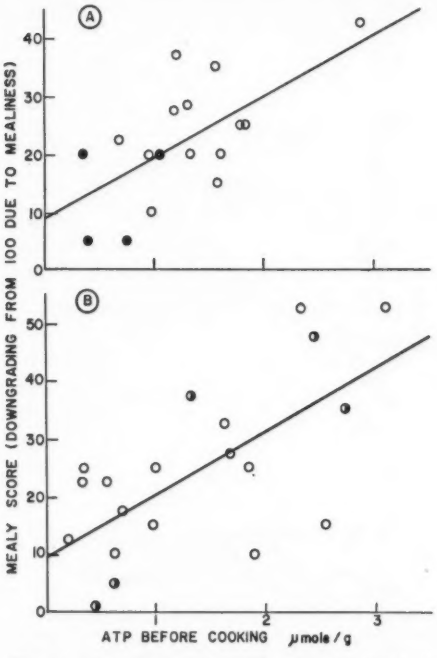

FIG. 4. Correlation of mealy score of individual fish and muscle ATP immediately before cooking. Filleted and frozen after 5 hr post-mortem iced storage ○; 10 hr ◑; 29 hr ●. A, fish caught June 28, 1966, $r = 0.62$, df = 15. B, July 28, 1966, $r = 0.66$, df = 18.

et al., 1966) of the trap fishing season. The high muscle glycogen values at killing (Table I) for the June 28 fish are consistent with poor production. However, even with such potentially poor material we have evidence that processing before rigor mortis can result in a product having a very satisfactory texture after extensive cold storage (36 weeks) at −23 C. Values for similar material processed in rigor (June 28) yielded the expected poor product (Table II).

TEXTURE, *p*H, AND THAW-DRIP RELATIONS IN ONCE-FROZEN FISH

Cod obtained in 1963 and 1964 (June 19–August 10) were examined after 9 months frozen storage and are reported in Fig. 7. Fish taken at the beginning and end of the season possess better frozen storage texture and produce less thaw-drip than those killed and frozen in midseason (Fig. 7).

In dealing with the *p*H and overall texture in individual fish rather than with the averages of individuals, difficulty was encountered in always obtaining similar clear-cut correlation. However, when the assessment of the texture

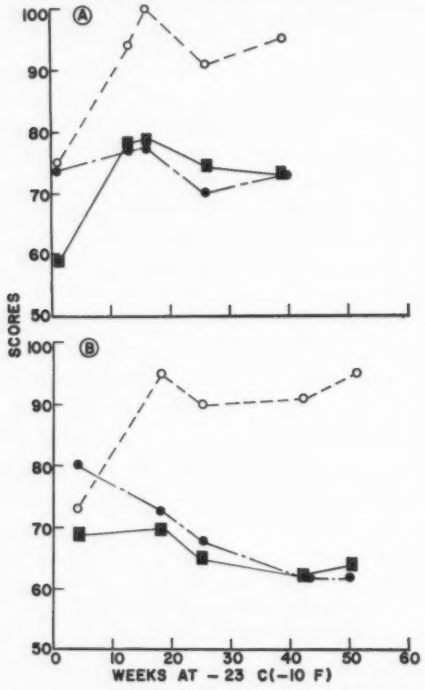

FIG. 5. Mealiness (○ – – – ○), toughness (● — – — ●), and overall texture (■ — ■) scores for fillets of prerigor fish frozen: A, June 19, 1964; B, June 21, 1965, and stored. A, each point represents the average of 18–24 assessments; B, each point at 4 weeks represents 311 observations; subsequent points are the average of 70–79 observations.

of all catches (June 4, 21, 28, July 19, 28, and August 2) taken in 1965–66 was based only on those characteristics which reflect toughness (thus scores were not downgraded for mealiness), excellent correlation was found between these features and the *p*H of individual fish at the time of cooking whether sampling took place immediately after freezing or after 6 and 9 months storage. Curves showing this relation for four randomly chosen lots of pre- and in-rigor frozen fillets are shown in Fig. 8. Thus, the overall frozen storage texture of Newfoundland trap fish varies with the period of catch (supporting earlier results, MacCallum et al., 1966) and patterns in quality can be predicted by thaw-drip and *p*H measurements at tasting.

COMMERCIAL AND CONTROLLED HANDLING OF ONCE- AND TWICE-FROZEN FISH

The taste panel found the June 29, 1962 once-frozen in-rigor controls, prepared as described earlier, to be better than the commercially handled

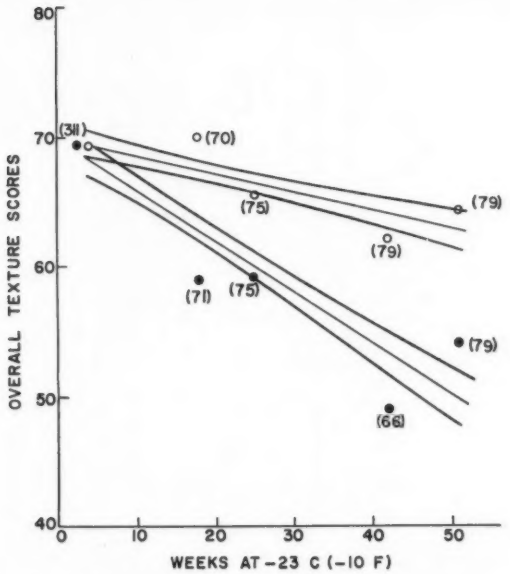

FIG. 6. Regression, with 95% confidence limits, of overall texture scores on time in frozen storage for once-frozen fillets of trap fish caught June 21, 1965. Open circles denote samples frozen pre-rigor using fish iced at the trap. Solid circles denote samples frozen from iced in-rigor fish. The number of observations for each average point is shown in parentheses.

samples at 16 weeks frozen storage (Table III) but in the case of the mid- and late-July lots the controls were no better at any period of cold storage than the fish handled without ice before freezing. The low texture scores of the July controls are a reflection of seasonal depreciation found in trap fish (of high glycogen content) iced 24 hr before freezing (MacCallum et al., 1966, 1967b).

Since work (Fig. 5 and 6) carried out on pre- vs. in-rigor freezing showed the superiority of the former, prerigor iced handling and freezing was compared in 1966 with commercial-type methods which provide for partial or complete resolution of rigor prior to freezing. Results of organoleptic and chemical assessment of once- and twice-frozen samples prepared as described earlier are given in Table IV.

The *p*H values for all once-frozen iced and non-iced fish and twice-frozen test samples were moderately low and quite uniform. Overall texture scores for once-frozen cod were lowest in the commercially handled fish prepared from the two catches and stored for 16 weeks or longer. The once-frozen packs originating from commercial-type handling were of borderline quality at

TABLE II. Taste-panel assessment of iced trap fish frozen before and during rigor in 1966.

Date of catch	Hr post-mortem filleted & frozen	State of rigor at freezing	0–2 weeks post-mortem				25 weeks post-mortem				36 weeks post-mortem			
			Texture	Taste	Overall accept-ability	N	Texture	Taste	Overall accept-ability	N	Texture	Taste	Overall accept-ability	N
June 28	5	Pre-rigor	61.8±1.01	62.6±1.38	61.2±1.03	99	56.9±1.32	49.6±1.68	56.4±1.34	39	59.5±1.30	41.5±1.61	54.0±1.28	55
	29	In-rigor	55.6±2.75	65.0±2.63	56.1±2.46	32					44.4±2.89	44.3±2.73	43.9±2.75	27
July 28	5	Pre-rigor	55.3±1.16	64.8±1.05	57.2±0.89	120	52.4±1.23	43.1±1.62	51.8±1.24	40	51.1±1.34	42.3±1.45	50.3±1.35	57

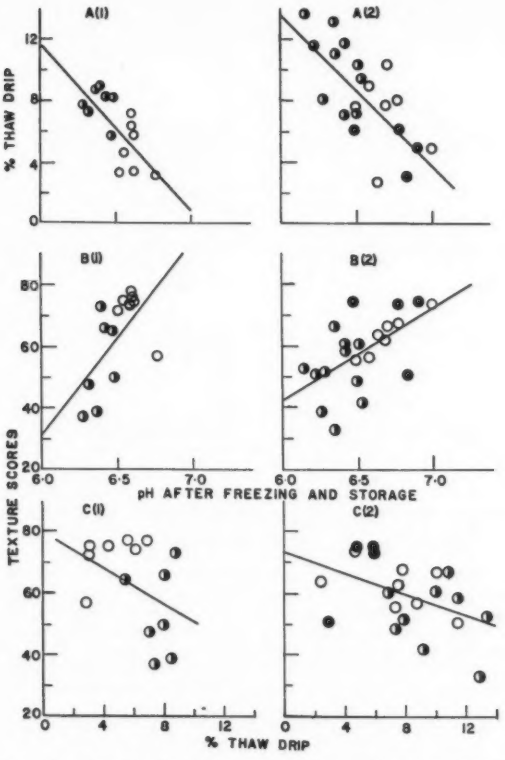

FIG. 7. Correlation found between three variables measured in once-frozen cold stored trap landings taken in 1963 and 1964: thaw-drip and *p*H (A) in the fish frozen (1) pre-rigor, (2) in-rigor; overall texture and *p*H (B) in the frozen fish and between overall texture and thaw-drip (C). Each point denotes an average value for three 1-lb packages. For each package eight observations were made of texture and one assessment of thaw-drip and *p*H. Fish from early "good" season ○; intermediate "poor" season ◑; and late "good" season ● were examined. Points cover frozen storage period from 0 to 9 months. A(1), $r = 0.70$, df = 12; A(2), $r = 0.71$, df = 19; B(1), $r = 0.60$, df = 12; B(2), $r = 0.58$, df = 20; C(1), $r = 0.42$, df = 12; C(2), $r = 0.47$, df = 19.

26 weeks, whereas those prepared from prerigor iced fish graded 13–19 points above the acceptability level, having changed little from the grade given the freshly frozen fish.

Immediately after refreezing, the twice-frozen fish, taken in June and July 1966 and given the various pre-freezing treatments described earlier, were as good as their once-frozen counterparts; however, the twice-frozen packs originating from prerigor iced raw material were better than the twice-

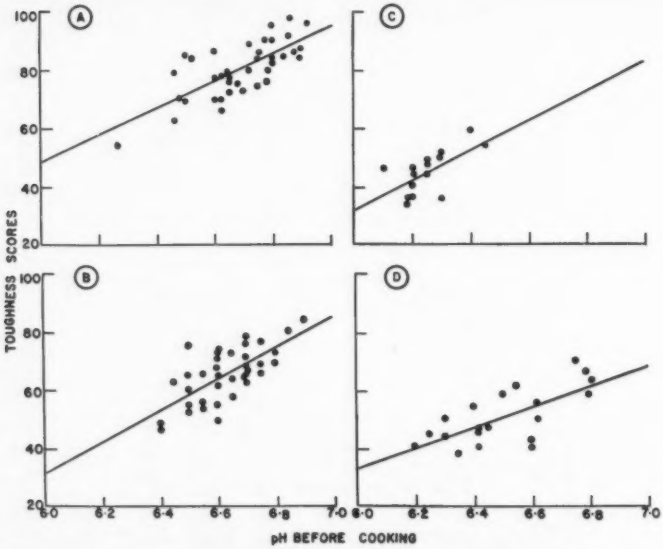

FIG. 8. Correlation between toughness score at fillet tasting and *p*H immediately before cooking for A, iced-at-sea fish, caught June 21, 1965, frozen pre-rigor, cold stored 1 month; B, tank-held fish (10 days), caught June 21, 1965, frozen in-rigor, cold stored 1 month; C, iced-at-sea fish, caught July 19, 1965, frozen in-rigor, cold stored 1 month; D, tank-held fish (4 days), caught August 2, 1965, frozen pre-rigor, cold stored 6 months. Each point represents the average of eight toughness assessments made on one fish and one *p*H value. Correlation coefficients are A, $r = 0.65$, df = 38; B, $r = 0.69$, df = 38; C, $r = 0.62$, df = 13; D, $r = 0.70$, df = 18.

TABLE III. Overall texture scores, as assessed by taste panels, during storage of once-frozen fillets prepared in 1962 from in-rigor iced fish or from fish after commercial type handling without ice.

Date of catch	Storage time (*weeks*)	Iced (24 hr) in-rigor control		Commercial handling			
				5½ hr uniced		10½ hr uniced	
		Texture (SE)	N	Texture (SE)	N	Texture (SE)	N
June 29	1	90±2.2	20	81±3.6	20	72±4.6	19
	8	90±2.4	15	90±5.5	15	87±3.9	15
	16	86±8.6	15	59±6.8	15	54±7.2	15
July 19	1	63±6.0	15	73±5.1	15	70±3.8	15
	9	66±4.8	15	79±2.7	15	69±5.4	15
	21	58±7.6	8	57±4.6	28	70±4.8	28
July 31	1	69±5.6	15	83±4.3	15	77±5.9	15
	8	67±6.1	15	74±6.1	15	67±6.3	15
	18	66±4.7	27	63±4.3	15	63±4.9	27
	24	65±4.6	24	69±4.5	15	63±4.4	24

TABLE IV. Taste-panel and chemical assessment during storage[a] of once- and twice-frozen fillets prepared in 1966 from prerigor iced fish or fish after

TABLE IV. Taste-panel and chemical assessment during storage[a] of once- and twice-frozen fillets prepared in 1966 from prerigor iced fish or fish after commercial-type storage without ice and after delayed icing.

Date of catch	Pre-freezing handling	Rigor at filleting	Post-freezing handling	Storage temp (C)	Storage time from capture (weeks)	Taste-panel assessment[b] Texture SE	Taste SE	N	pH	Thaw drip	EPN	FFA (mg/100 g)
June 29	Iced 3 hr filleted	Pre-rigor		−23	1	63±2.12	74±1.74	24	6.44[e]	10.2[d]	2.06[d]	137[e]
					16	61±3.18	61±3.64	21	6.28[e]	5.0[d]	1.88[d]	354[e]
					26	59±2.04	48±2.18	22	6.47[e]	5.6[f]	–	297[e]
					42	57±1.49	38±1.99	39	–	–	–	389[e]
	Iced 3 hr	Pre-rigor	Block-thawed 16 weeks post-mortem, filleted, refrozen	−23	16	62±2.05	65±2.08	21	6.18[e]	16.0[d]	1.87[d]	358[e]
					26	48±2.49	51±2.11	24	6.34[e]	13.0[f]	–	299[e]
	Uniced 9 hr at 10–15 C ambient, filleted	Incipient to full			1	60±2.70	72±2.08	24	6.33[e]	14.4[d]	1.94[d]	165[e]
				−23	16	43±4.12	51±1.69	22	6.29[e]	14.4[d]	1.58[d]	399[e]
					26	44±1.91	39±2.27	24	6.50[e]	7.3[f]	–	332[e]
	Uniced 9 hr	Incipient to full	Block-thawed 16 weeks post-mortem, filleted, refrozen	−23	16	56±1.77	63±1.87	22	6.46[e]	13.9[d]	1.49[d]	329[e]
					26	44±1.96	45±2.24	23	6.30[e]	11.8[f]	–	296[e]
	Uniced 9 hr, iced 15 hr, filleted	Partly resolved		−23	1	52±2.34	64±2.85	25	6.36[e]	13.8[d]	2.14[d]	147[e]
					16	54±3.93	63±1.99	24	6.28[e]	12.9[d]	2.00[d]	347[e]
					26	35±1.68	38±2.17	24	6.29[e]	9.9[f]	–	318[e]
	Uniced 9 hr, iced 15 hr	Partly resolved	Block-thawed 16 weeks post-mortem, filleted, refrozen	−23	16	46±3.43	61±2.36	22	6.28[e]	15.2[d]	1.72[d]	292[e]
					26	37±2.23	45±2.33	24	6.43[e]	14.6[f]	–	285[e]
				Block −23 / Fillets −40	26	45±2.07	53±1.98	23	6.23[e]	12.9[f]	–	302[e]
				Block −46 / Fillets −40	16	52±2.25	63±1.97	22	6.37[e]	13.6[d]	1.91[d]	366[e]
					26	47±2.48	47±2.64	24	6.39[e]	11.3[f]	–	252[e]
July 27	Iced 3 hr filleted	Pre-rigor		−23	1	54±2.48	72±2.10	24	6.20[e]	7.8[d]	1.98[d]	163[e]
					16	49±2.29	48±2.24	24	6.34[e]	5.3[d]	2.00[d]	356[e]
					26	53±2.21	47±2.90	19	6.46[e]	10.9[f]	1.85[e]	275[e]
					38	45±1.34	37±1.29	38	–	–	–	296[e]

(Continued)

TABLE IV. Taste-panel and chemical assessment during storage[a] of once- and twice-frozen fillets prepared in 1966 from prerigor iced fish or fish after commercial-type storage without ice and after delayed icing. — (*Concluded*)

Date of catch	Pre-freezing handling	Rigor at filleting	Post-freezing handling	Storage temp (C)	Storage time from capture (*weeks*)	Taste-panel assessment[b] Texture SE	Taste SE	N	pH	Thaw drip	EPN	FFA (*mg/100 g*)
July 27	Iced 3 hr	Pre-rigor	Block-thawed 16 weeks post-mortem, filleted, refrozen	−23	16	53±2.97	54±2.00	24	6.29[e]	13.2[d]	1.80[d]	399[e]
					26	42±2.78	41±2.74	17	6.21[e]	16.8[f]	1.48[e]	353[e]
	Uniced 9 hr at 12–15 C ambient, filleted	Incipient to full	-	−23	1	43±2.55	64±2.54	24	6.26[e]	13.3[d]	1.77[d]	146[e]
					16	36±2.75	48±2.12	24	6.25[e]	14.9[d]	1.87[d]	307[e]
					26	34±2.88	36±2.86	17	6.26[e]	16.8[f]	1.73[e]	275[e]
	Uniced 9 hr	Incipient to full	Block-thawed 16 weeks post-mortem, filleted, refrozen	−23	16	46±1.83	50±2.22	24	6.48[e]	15.3[d]	1.99[d]	362[e]
					26	38±2.40	44±1.58	18	6.50[e]	12.1[f]	1.35[e]	283[e]
	Uniced 9 hr, iced 15 hr, filleted	Partly resolved		−23	1	61±3.01	73±2.29	24	6.29[e]	9.0[d]	1.89[d]	164[e]
					16	42±2.67	45±2.24	24	6.24[e]	12.8[d]	1.85[d]	396[e]
					26	41±2.33	46±2.08	16	6.38[e]	11.6[f]	1.67[e]	259[e]
	Uniced 9 hr, iced 15 hr	Partly resolved	Block-thawed 16 weeks post-mortem, filleted, refrozen	Block −23 / Fillets −23	16	45±1.94	56±1.76	24	6.31[e]	13.5[d]	1.58[d]	327[e]
					26	39±3.29	50±2.55	17	6.44[e]	15.4[f]	1.54[e]	278[e]
				Block −23 / Fillets −40	26	42±2.99	43±3.34	14	6.45[e]	13.7[f]	1.75[e]	269[e]
				Block −46 / Fillets −23	26	48±2.50	45±2.48	16	6.55[e]	12.7[f]	1.62[e]	218[e]
				Block −46 / Fillets −40	16	50±2.44	54±3.11	24	6.60[e]	18.6[d]	2.03[d]	189[e]
					26	53±1.29	53±2.19	17	6.42[e]	19.1[f]	1.92[e]	178[e]
					38	48±2.06	43±2.02	32	–	–	–	166[e]

[a]Blocks of headed, eviscerated fish frozen after delayed icing were stored at either −23 C (−10 F) or −46 C (−50 F). The fillets processed from them were stored at −40 C (−40 F). Both once- and twice-frozen samples of other treatments were stored at −23 C (−10 F).

[b]Average overall texture and taste scores of three 1-lb packages each consisting of parts of more than one fillet are followed in each case by the standard error of the mean. The number of observations (N) applies to the two assessments.

[c]Single random sampling of each of two 1-lb packages consisting of more than one fillet, results averaged.

[d]Duplicate random sampling of each of two 1-lb packages consisting of more than one fillet, results averaged.

[e]Single random sampling of each of three 1-lb packages consisting of more than one fillet, results averaged.

[f]Duplicate random sampling of one 1-lb package consisting of more than one fillet, results averaged.

frozen lots handled in commercial fashion. The texture in the twice-frozen material prepared from iced, prerigor frozen fish dropped significantly (from 62 to 48, June samples; and from 53 to 42, July samples) to an above-acceptable level after a further storage period of 10 weeks, making it little better than the twice-frozen product of commercial-like handling. The latter results are not surprising in view of previous findings reported in this paper that once-frozen "prerigor" fish, although undergoing rigor-like biochemical reactions, have some potential for extended frozen storage whereas fish of the same catches, if allowed to proceed into rigor, have much less. It would appear that during thawing and refreezing or during subsequent frozen storage, rigor was resolved in the prerigor frozen cod used for double freezing. Although quality was not affected immediately by thawing and refreezing, there were rapid changes after further cold storage.

Dramatic improvement (Table IV) was realized by storage of both dressed fish and refrozen fillets at very low temperatures (−46 and −40 C, respectively). In the July 27 catch low holding temperatures apparently prevented the textures and EPN values in refrozen stored samples of commercial-like handling from dropping below those in once-frozen prerigor controls stored at −23 C. This held true (texture scores alone reported) even after 38 weeks frozen storage. A similar effect, though not so pronounced, was noted in the June 29 samples. It would appear essential to store the blocks of unprocessed material at the very low temperature to realize these benefits in quality, but perhaps not so essential to store the refrozen fish of such lots below −23 C if the fish is to be consumed within a few weeks from the time of refreezing. On the other hand, if the unprocessed blocks are held at −23 C, subsequent holding of the refrozen fillets at −40 C offers no clear-cut improvement over storage of both whole and processed fish at the higher temperature. Refrozen fillets (June samples) stored at −23 C were significantly (5%) poorer, as assessed by the texture score at 10 weeks, than refrozen fillets stored at −40 C, both having been prepared using blocks stored at −23 C. However, there was no significant difference in refrozen storage quality of fillets of July fish prepared from similarly stored blocks when the fillets were stored at the two different temperatures.

Peters et al. (1968) report FFA values in Sable Island cod to increase sharply as a result of thawing, refreezing, and again with storage of the refrozen product at −18 C (once-frozen control fillets stored at −29 C). In this present study, FFA values for fish from the two trap landings stored at −23 C were high (of the same magnitude as reported previously (MacCallum et al., 1964b)) and showed no difference due to thawing, refreezing, or to further storage. With these trap fish there was no effect on FFA values of pre- vs. in-rigor freezing. The FFA values for both June and July catches at 26 weeks frozen storage were lower for those fish stored at −46 C as unfilleted block fish and at −40 C as refrozen fillets than for those stored at −23 C in both forms.

Extractable protein nitrogen in the July samples handled without ice and once-frozen was lower (5% significance) after 16 weeks frozen storage than

[d]Duplicate random sampling of each of two 1-lb packages consisting of more than one fillet, results averaged.
[e]Single random sampling of each of three 1-lb packages consisting of more than one fillet, results averaged.
[f]Duplicate random sampling of one 1-lb package consisting of more than one fillet, results averaged.

in the prerigor once-frozen fish but there was no significant difference between the fish of different pre-freezing treatment after 26 weeks frozen storage. Statistical analysis of the June and July once-frozen samples combined, independent of treatment before freezing, show correlation (5% significance) between thaw-drip and overall texture scores and between EPN values at tasting and the overall texture scores but not between EPN values and overall acceptability scores. With the twice-frozen samples (three pre-freezing treatments combined) there was also significant correlation (5%) between EPN values at tasting and the overall texture scores and between EPN and overall acceptability scores for the July catch, based on analyses and panel results at 16 and 26 weeks. Peters et al. (1968) reporting on twice-frozen Sable Island cod stored at −18 C show correlation (5% significance) between EPN and overall acceptability scores but no correlation for once-frozen controls stored at −29 C.

With the present once-frozen samples there was correlation (1% significance) between EPN and FFA values for the June fish examined after 1 and 16 weeks frozen storage (Fig. 9) but no correlation between the same

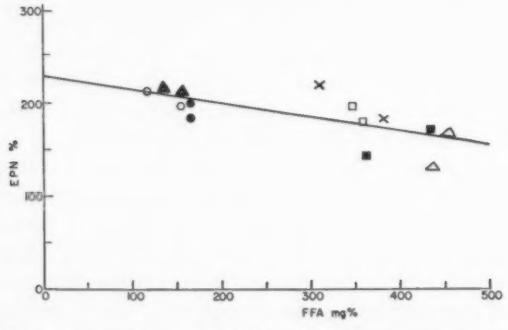

FIG. 9. Correlation of EPN and FFA values in once-frozen fillets of June (1966) trap-caught cod examined after 1 and 16 weeks frozen storage (at −23 C except where noted). Circular symbols and solid triangles represent samples examined at 1 week, other symbols 16 weeks. Prerigor frozen ○, □; 9 hr uniced ●, ■; 9 hr uniced followed by 15 hr iced ▲, × (and △ at −40 C). $r = 0.72$, df = 13.

variables in the case of the July samples examined after 1, 16, and 26 weeks frozen storage. There was no correlation between EPN and FFA values for the combined June and July twice-frozen fish of similar treatment (all three treatments assessed separately) or between the same variables for fish of all treatments combined. There was correlation (1% significance) of the combined data (Fig. 10) for the June and July once-frozen samples between FFA values and strictly taste scores (the data considered separately for the two catches also showed correlation (1% significance)) but no correlation between FFA

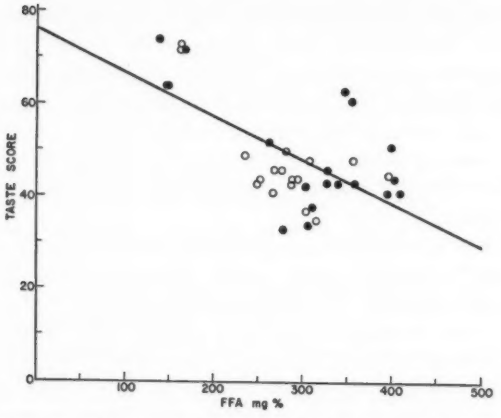

FIG. 10. Correlation between FFA values and taste scores for the June and July (1966) once-frozen fillets of trap-caught cod assessed after 1, 16, 26, and 40 weeks storage. June fish ●; July fish ○. $r = 0.63$, df = 35.

values and overall texture scores. For twice-frozen fish there was no correlation between FFA values and taste, overall texture, and overall acceptability for any particular treatment or for all treatments combined. Peters et al. (1968) found no correlation between FFA values and overall acceptability scores for the once-frozen Sable Island cod referred to previously but did find a correlation (5% significance) between the data obtained for variously treated twice-frozen fish.

The thaw-drip was less in the once-frozen prerigor samples (Table IV) than in the once-frozen commercial-type samples. With twice-frozen fish there was no difference in thaw-drip with pre-freezing handling.

Filleting these inshore fish after the resolution of rigor is avoided when possible, due to the soft flaky condition of the fillet and the low yield of first-class material. To illustrate, the June 29, 1966 control fish described earlier, filleted before the onset of rigor, all yielded firm, whole fillets. Eighty per cent of the catch which was left uniced at the ambient temperature of 10–15 C was in rigor at 9 hr. Half the fish were then filleted, yielding only 27% good, firm fillets, the remainder being soft and flaky and suitable for a second quality pack. At the same time the remaining fish were well iced and left for 15 hr before filleting. Forty-five per cent of the fish were still in rigor at filleting, rigor being partially or completely resolved in the remainder; 13% of the fillets were soft, the remaining 87% very soft and flaky or mushy and suitable for a second quality pack.

The results show the desirability of quick chilling followed by a single freezing of prerigor material and storage at −23 C, particularly in fish handled in July, or of storage at −46 C in fish handled prior to freezing under conditions presently applying to commercial processing. A poor product, being one of

barely tolerable quality, was obtained by single freezing fish that were handled without ice before freezing and were subsequently stored at –23 C. It would appear that single freezing the fillets of these chilled cod before rigor ensures maintenance throughout the required storage period of the quality at freezing. However, it is fairly certain that industry could handle only a portion of the catch pre-rigor, even if geared for the purpose, and to obtain the next best once-frozen product should use chilled-on-the-bone, in-rigor material for filleting and freezing.

The next best product to be obtained from prerigor, chilled material results from double-freezing. Here again, industry could handle only part of the catch in this manner. With fish frozen pre-rigor and stored at –23 C, one would expect the immediate refrozen quality to be equal to the quality of once-frozen prerigor material packed at the same time for as long a period as 16 weeks after killing. A fairly rapid decrease in quality occurs in the re-frozen material in the 10 weeks after refreezing. Thus, it would appear that thawing and refreezing the prerigor blocks should be delayed until markets for the reprocessed fish are assured.

A very satisfactory twice-frozen product can be obtained also by holding the unfilleted blocks of non-iced, gutted fish at –46 C before thawing and re-freezing, followed by remaining storage at –23 C (or –40 C). However, such low-temperature storage is presently beyond the scope of commercial operations.

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Marine Mortality Schedules of Pink Salmon of the Bella Coola River, Central British Columbia[1]

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ABSTRACT

The hypothesis that natural mortality rates of pink salmon during early life are generally much larger than during the later period has been tested for 3 brood-years of the Bella Coola River stock using a two-stage marking technique. Average daily losses to the population during the first 40 days are estimated to vary between 2 and 4%, and for the later 410-day period between 0.4 and 0.8%. These rates produce losses amounting to between 59 and 77% of the initial population during the first 40 days. Of the population surviving at 40 days, further losses of between 78 and 95% occurred. The latter losses are considered to be maximum estimates because of bias introduced by catches of unknown magnitude. Thus, although the early mortality is exceeded by the later, the time period is approximately 10 times as long, and the intensity of the mortality rate is much higher during early sea life.

INTRODUCTION

Marine natural mortality rates of pink salmon (*Oncorhynchus gorbuscha*) have received little study until recent years. The general level of loss, from the time a year-class entered the sea as fry to the time of return as adults, has been about 95% for central British Columbia stocks (Neave, 1953; Hunter, 1959). From comparisons of fecundity and total survival among pink salmon and chum salmon (*O. keta*) (the two species approaching ecological similarity but with dissimilar age structures) it was proposed that an initial short period (termed "coastal") of high mortality rate was followed by a relatively long period (termed "oceanic") of low mortality rate (Parker, 1962a, b). This hypothesis seemed acceptable on the grounds that unprotected young generally are subjected to initially high mortality rates which decline as the animal grows and becomes better able to cope with its environment. This, however, does not establish the validity of the hypothesis in the specific application to pink salmon.

Two attempts to test this hypothesis by indirect measurement of prevailing mortality rates on a particular stock of central British Columbia pink salmon have been described. The first attempt (Parker, 1964) failed to achieve its purpose. The second attempt (Parker, 1965) resulted in the conclusion that, for the 1961 brood-year Bella Coola stock, during the initial 40-day coastal period approximately 77% of the entering population had died from natural causes. Of those yet alive at 40 days, approximately 78% died from natural causes during the following oceanic stage, a period of about 410 days.

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The assumptions necessary to arrive at these estimates precluded placing any great emphasis on these "most probable" values, yet the average instantaneous mortality rates on a per day basis (coastal, 0.0363; oceanic, 0.0037) were in different orders of magnitude and the difference could not reasonably be accounted for either by statistical error or known bias. Thus the hypothesis was supported by this result.

Data are now available for 2 additional brood-years of pink salmon originating in streams associated with the Bella Coola River. It is the purpose of this report to present and analyse these data from the standpoint of further testing the hypothesis on the distribution of marine natural mortality rates. Other aspects of the vital statistics of these populations are included. These, however, are a by-product of the main area of concern.

NOTATION

N: denotes the number of fish in any specified population.

ARV: a subscript denoting the adipose–right ventral fin mark.

ALV: a subscript denoting the adipose–left ventral fin mark.

t: a subscript specifying a particular day or span of days to which the quantity is referred.

S: denotes the fraction of a population surviving during a specified time period from natural causes of death.

S_M: denotes the fraction surviving the short-term effects of marking.

m: denotes the continuing long-term marking coefficient which modifies the instantaneous natural mortality rate (Parker et al., 1963). Herein m is considered as a constant measured without error, although this is not strictly true. Its value for this application is taken as 1.38 (Parker, 1965).

q: denotes the instantaneous natural mortality rate on a per day basis. It is considered as a variable.

c: a subscript referring to the coastal period of life history, herein considered as of 40 days duration, i.e. from $t = 0$ to $t = 40$.

o: a subscript denoting the oceanic period, herein considered as the time period from $t = 40$ to $t = 450$, the latter being approximately the time of peak abundance of the adult salmon in the commercial fishery at the coast.

The use of 40 days for the coastal period is arbitrary and only for the purposes of this paper. The real coastal period may extend over a longer period of time, with the remaining oceanic period being of shorter duration than the herein specified 410 days.

EXPERIMENTAL DESIGN

The general approach used here is elaborated from that presented by Taguchi (1961) and may be stated as follows:

$$\text{let } N_k = N_i S_M \exp -m(k-i)\bar{q} \qquad \ldots\ldots(1)$$

where the marked population is released at $t = i$ and enumerated again at $t = k$. We may also have two populations of distinctively marked fish, N_a and N_b, the one at liberty for an intermediate time period, j–k. Two equations may be written

$$\left.\begin{aligned} N_{ak} &= N_{ai} S_{Ma} \exp -m(k-j)\bar{q}_2 - m\,(j-i)\bar{q}_1 \\ N_{bk} &= N_{bj} S_{Mb} \exp -m(k-j)\bar{q}_2 \end{aligned}\right\} \ldots\ldots(2).$$

By solving these equations simultaneously the difference in the fractions surviving of the two populations (expressed by the exponents) may be derived.

Thus

$$\exp -m(j-i)\bar{q}_1 = \frac{N_{ak}}{N_{bk}} \frac{N_{bj}}{N_{ai}} \frac{S_{Mb}}{S_{Ma}} \quad \ldots\ldots(3)$$

is an estimate of S_{ij} provided the $m\bar{q}_1$'s are equal. In the present application several assumptions must be satisfied. These will be listed as encountered.

Assumption 1.—The marking coefficient m is either identical or known for both groups.

Assumption 2.—The natural mortality rate is identical for both groups during the period j–k.

The fraction of the *marked* population surviving the time interval between release dates of the two populations is, then, estimated from three ratios:

$$N_{ak}/N_{bk},\ N_{bj}/N_{ai},\ S_{Mb}/S_{Ma}$$

and it is not necessary to know the absolute values of these parameters if the ratios can be established from sample statistics. This method can easily be extended to estimate survival rates for any sequential series of time periods by increasing the number of marked populations.

This model has been used to estimate the survival of pink salmon fry of the Bella Coola River (Fig. 1) during their coastal and oceanic stages. The general features of their movements and migration routes in coastal waters, both for juveniles and adults, has been described (Parker, 1965). Briefly, most of the fry enter the estuary during the latter half of April. As they grow they move slowly down Burke Channel until at 30–40 days they enter Fitz Hugh Sound. At the end of May they form into migrating schools and move into the open waters of Queen Charlotte Sound. The seaward routes followed from Fitz Hugh Sound vary among the years. In some years (1961, 1962) the bulk of the population appeared to migrate through Nalau Pass. The succeeding year (1963) the population was not observed in significant numbers after it entered Fitz Hugh Sound. In 1964 the population appeared to linger in Fisher Channel, with significant migration through Johnson and Return channels to Milbanke Sound. In 1965 the population appeared to have moved seaward mainly through Lama Pass and Hunter Channel.

Two marks have been used, ARV and ALV, which are a priori considered to satisfy Assumption 1. The second marking has been applied, as nearly as possible, to fish associated with the main centre of abundance of the original marked population. Thus, in general, Assumption 2 is thought to be justified; however, it will be discussed further in relation to specific interpretations.

Previous experience has shown the futility of estimating the ratio N_{ak}/N_{bk} from sample statistics in this specific application. At the time of the second

marking, stocks from streams other than Bella Coola are mixed in with the target stock, and upon return these stocks are subjected to different rates of exploitation. Thus it has been necessary to estimate the total return of both marked populations involving both catch and escapement estimates.

Assumption 3.—The values of S_{Ma} and S_{Mb} are both assumed to be equal to unity.

The evidence supporting this assumption has been reviewed in an earlier paper (Parker, 1965). It is sufficient to say here that losses to the marked populations after release appear to be associated with long-term natural mortality rates through the agency of predator selectivity rather than death arising from the treatment per se. We have repeatedly held marked and unmarked lots together, after release of the main population, for periods up to 6 weeks' duration without significant differences in survival.

Each estimate of a term used in equation 3 has statistical variance. Variance of each component has been combined according to methods given by Deming (1943). In some cases estimates have been made by extrapolation or other means which do not provide a direct estimate of the variance. In these cases I have subjectively estimated the variance by first setting arbitrary boundaries to the estimate by whatever clues were available. These arbitrary boundaries have been equated to a 50% confidence interval, i.e., it is the author's *opinion* that the boundaries stated stand a 50–50 chance of enclosing the true value being estimated. Thus the estimate takes the form of a statement of probable error, i.e., $\bar{x} \pm 0.6745\ \sigma$. From this estimate the σ^2 is estimated. This statement of probable error assumes that the distribution is symmetrical and that 50% of the error is contained within defined limits.

In all other cases the variance has been calculated either as the binominal approximation, $\hat{\sigma}^2 = npq$ or, where p approaches 0 or 1.0, the Poisson approximation $\hat{\sigma}^2 = \bar{x}$.

Several sources of bias were present and will be noted and discussed in conjunction with the affected estimates.

1962 BROOD-YEAR

Marked Juveniles

On April 21, 1963, approximately 500,000 pink salmon fry were seined in the vicinity of Whiskey Bay in North Bentinck Arm (Fig. 1). These were transported to holding pens alongside the floating laboratory. During the period April 22–27 fry were marked, under anaesthesia (tricaine methanesulfonate), by amputation of the adipose and right ventral fins (ARV). After recovery, the marked fish were returned to the holding pens and accumulated there until the completion of marking. The entire population was released on April 28.

During each day of marking, samples were taken from each marker's production and examined to establish quality control. Sample variances were calculated and combined into the estimate of the total marked population released. This estimate and its variance are: $\hat{N}_{0\cdot ARV} = 166{,}862$, $\hat{\sigma}^2 = 696{,}688$.

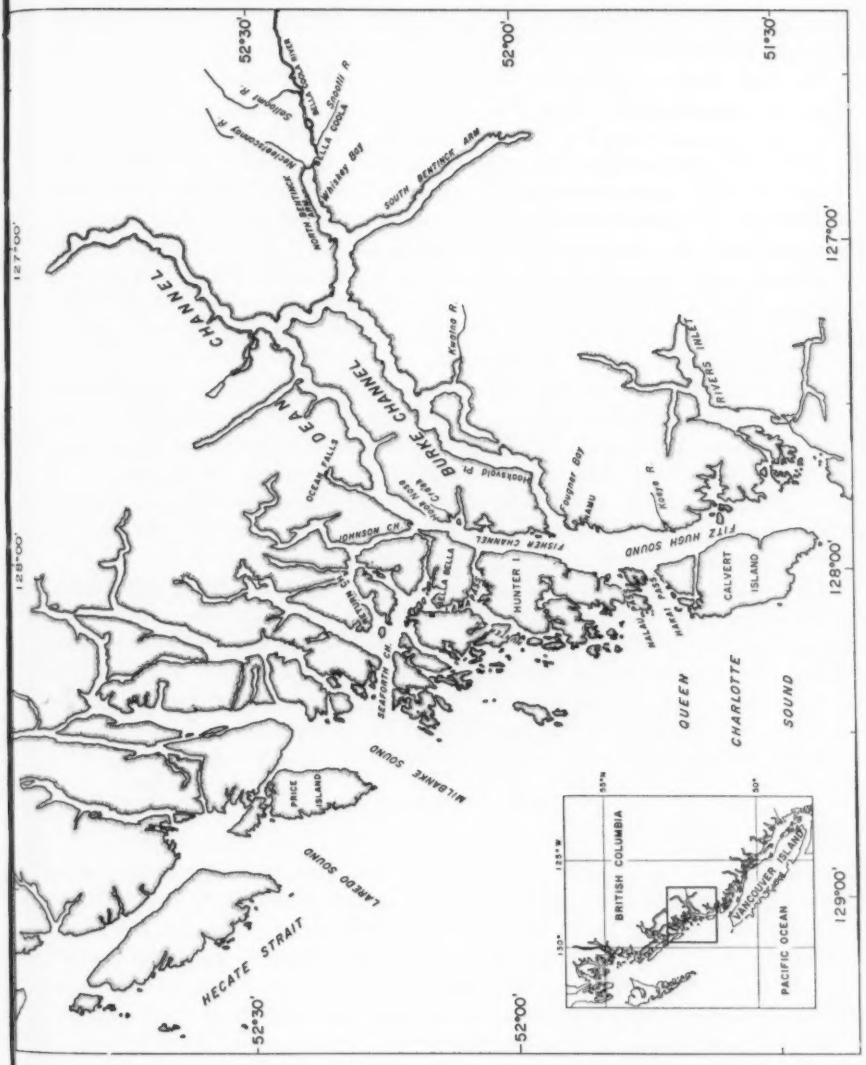

FIG. 1. Central area of the British Columbia coast.

After the release of the ARV population, accumulations of juvenile pink salmon were sampled daily and examined for marked members. In this manner, the marked population was observed to spread out and mix with the unmarked population and to slowly move westward into and through Burke Channel.

It was expected that a large proportion of the Bella Coola population would continue in a southwest direction in Fitz Hugh Sound and pass through

Nalau Pass into Queen Charlotte Sound. Accordingly, the laboratory was anchored in Nalau Pass in preparation for a second marking of the population.

No large body of juvenile pink salmon arrived at Nalau Pass. Fish were seined and marked ALV, using the same technique outlined above, but each day's marking was released on the following day. The first release was on May 30. It became obvious that only a small number of fish were present, for the incidence of ALV marks increased to about 50% of the catch after the release of 65,000 fish. Marking was suspended after the third day. On June 6 schools of juvenile pink salmon appeared again in Nalau Pass. A number were captured and releases were made from June 7 to June 9. The estimated total release is as follows: $\hat{N}_{38\cdot ALV} = 153{,}237$, $\hat{\sigma}^2 = 266{,}166$. As indicated in the subscript above, the date when 50% of the ALV population was released was 38 days after the release of the ARV population.

A number of mismarks (any other than ARV or ALV) were also released from both markings. These will not be considered in enumerations of the returning adults, for the same mismarks are common to both markings. Some alteration, primarily of ARV to adipose-both ventral might be expected from natural causes, e.g. chalimus larvae of the parasitic copepod *Caligus clemensi* (Parker and Margolis, 1964), which would bias the estimate of S_{ij}, the fraction of the population surviving during the early sea life period.

Assumption 4.—This bias is assumed to be negligible. After the release of the marked population, nearly 1000 ARV marked fish were recaptured and examined before the second marking. In no case was the left ventral fin either attacked or mutilated by an agent attributable to this source. Although the bias may exist, it must be so small as to not significantly affect the estimation of mortality rates of the ARV population. Its possible effect is to bias the estimation of coastal mortality in the direction of being too large.

1964 ADULT RETURN

Commercial Fishery Catch

Returning adult pink salmon of the Bella Coola population are known to be included, to some degree, in catches from statistical areas 6, 7, 8, and 9. These areas are outlined in Fig. 2. In 1964, 93.9% of the total catches of areas 7 and 8 were taken during a 6-week period in July and August. During this period, from July 12 to August 22, catches landed at Namu and at Vancouver were sampled to estimate the incidence of marked fish. Consistent information was obtained only from catches originating in areas 7 and 8. This deficiency causes a significant bias in the results and will be discussed later.

Official catch statistics, published by the Department of Fisheries, state catches originating within the broad statistical areas. The area 8 Conservation Officer, by daily contacts with fish-buying and fish-packing vessels, made field estimates which separate area 8 weekly catches into those originating in the Fisher Channel-Fitz Hugh Sound purse-seine and gillnet area and the Bella Coola gillnet area comprising upper Burke Channel and North Bentinck Arm. In addition, catches landed by the relatively unimportant and

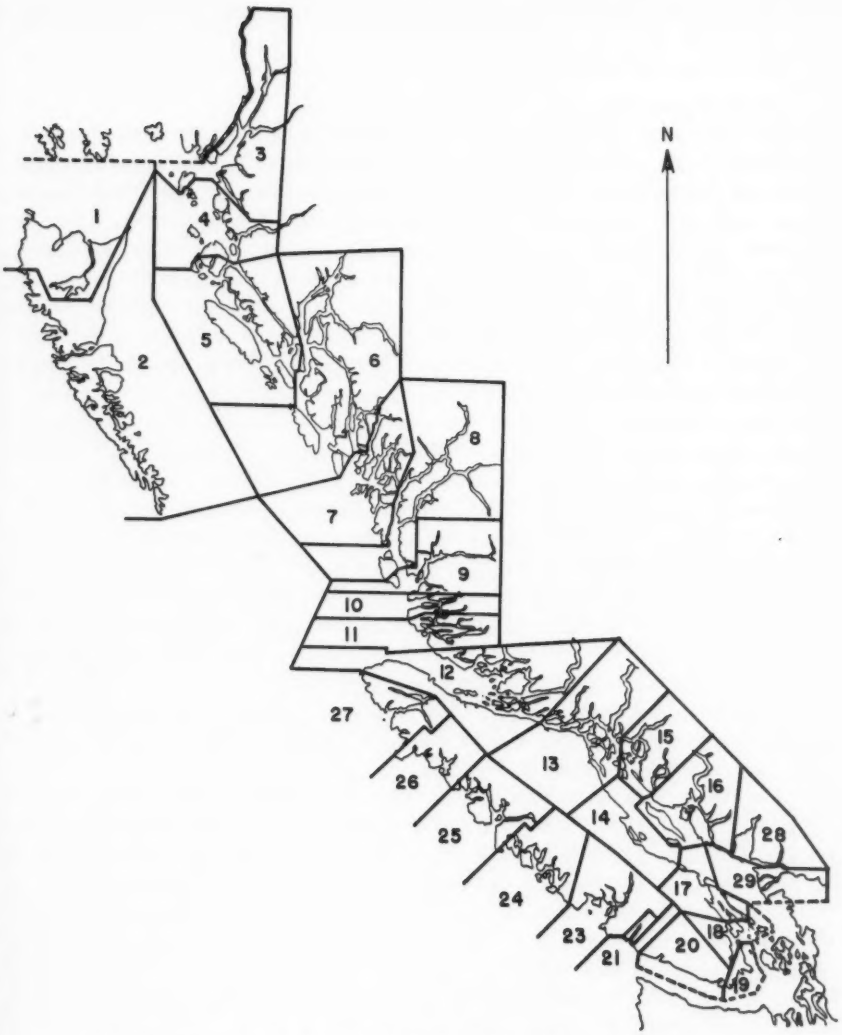

FIG. 2. Division of British Columbia coastal area into statistical areas.

sporadic gillnet fishery in Dean Channel were estimated. These field estimates for the gillnet catches were 98.9% of the total official catch figure. For purposes of subdividing the area 8 catch, the field estimates for the Bella Coola and Dean Channel catches are used and subtracted from the official area 8 gillnet catches to estimate the portion originating in the Fisher Channel–Fitz Hugh Sound area.

Gillnet and purse-seine catches were next combined within the Fisher Channel–Fitz Hugh Sound and area 7 fisheries as statistical tests disclosed

no evidence of significant differences in the incidence of either mark in weekly samples from these two types of gear [$\chi^2_{ARV} = 2.490$, df = 4, $P \approx 0.64$; $\chi^2_{ALV} = 4.582$, df = 6, $P \approx 0.60$].

Troll catches were not sampled.

Assumption 5.—The incidence of both marks in the troll catches was not significantly different from that of the net fisheries within the same statistical areas and fishing weeks. Direct evidence supporting this assumption is lacking. It can only safely be stated that there may have been similarity. To ignore the troll catches would increase the bias mentioned earlier from the lack of samples from area 6 and 9 catches. This bias has the effect of assigning fishing mortality to natural mortality, thus increasing the estimate of the ocean component. To include the troll catches with the net catches may have the opposite effect, for the troll catches largely come from the outer coasts bordering Queen Charlotte Sound and, therefore, the probability of including fish from non-area 8 streams is increased. The troll catch from area 8 was small, amounting to only 0.07% of the area 8 total; therefore, the bias from this source is negligible. The troll catch in area 7, however, was 9.8% of the total area 7 catch and if included it might introduce a significant bias which would partially offset that caused by the lack of area 6 and 9 catches. It is concluded that although Assumption 5 is not justified with evidence, including troll catches results in an improved estimate of the number of marked fish caught. Hence, the troll catches from area 7 have been included with those of the area 7 net fisheries and troll catches from area 8 have been included with the Fisher Channel–Fitz Hugh Sound net catches.

With these adjustments the 1964 commercial catches for areas 7 and 8, the sampling data and the estimates of catches of ARV and ALV marked pink salmon are presented in Table I.

Two features are immediately evident. The Bella Coola gillnet catches contained members of both marked populations thus indicating that substantial numbers of the Bella Coola stock had been marked ALV. Fish belonging to other stocks, presumably spawning in local Fisher Channel–Fitz Hugh Sound streams had also been marked ALV. As the relatively large Bella Coola stock moved out of area 7 and Fisher Channel–Fitz Hugh Sound fishing areas, catches diminished and ARV marked fish became scarce. At the same time the incidence of ALV marked fish increased. These observations confirm those of previous reports (Parker, 1964, 1965).

A second serious deficiency exists in the sample data: the lack of samples during the pre- and post-sampling periods. Catches during these periods were relatively small. During the pre-sampling period they were unimportant in that the small error introduced would be very little biased in respect to ARV: ALV ratio. In the post-sampling period, however, the ARV: ALV ratio undoubtedly changed. To ignore these catches the estimate of the ALV return would be significantly underestimated, whereas the ARV estimate would be little, if at all, affected. This error would bias the estimate of coastal mortality in the direction of being too small. It is, therefore, necessary to estimate both the

TABLE I. Estimated numbers of ARV and ALV marked pink salmon taken by areas 7 and 8 fisheries in 1964.

		Catch (*pieces*)	Sample (*pieces*)	Observed		Estimated			
				ARV	ALV	ARV	σ^2ARV	ALV	σ^2ALV
Area 7									
	Pre-	10,433	Nil	–	–	8[a]	–	11[a]	–
July 12–18	1	19,587	2,012	2	3	51	514.1	41	411.7
July 19–25	2	44,372	4,295	3	1				
July 26–Aug. 1	3	98,871	6,786	5	7	73	1,061.4	102	1,486.0
Aug. 2–8	4	180,284	46,399	5	20	19	75.5	78	301.9
Aug. 9–15	5	160,808	37,460	1	17	5	27.2	73	313.1
Aug. 16–22	6	106,551	13,727	–	9			70	542.1
	Post-	74,967	Nil	–	–	0[b]	–	65[b]	–
Fisher–Fitz Hugh									
	Pre-	67,338	Nil	–	–	36[b]	–	44[b]	–
	1	588,955	141,145	84	117	351	1,461.6	488	2,035.8
	2	896,687	216,049	139	227	577	2,394.1	942	3,909.8
	3	389,761	78,855	56	91	277	1,367.3	498	2,222.0
	4	179,359	38,247	20	104	94	439.9	488	2,287.3
	5	97,104	35,982	6	157	16	43.7	424	1,143.9
	6	85,324	40,382	3	189	6	13.4	399	842.6
	Post-	58,506	Nil	–	–	0[b]	–	291[b]	–
Bella Coola[c]									
	Pre-	4,857	Nil	–	–	4[d]	–	6[d]	–
	1	7,580[e]	3,130	1	–	2	5.9	–	–
	2	160,363	51,929	44	68	136	419.9	210	648.9
	3	208,276	78,439	64	102	170	452.0	271	720.4
	4	100,729	58,775	53	77	91	155.3	132	225.7
	5	Closed	–	–	–	–	–	–	–
	6	5,455	4,047	2	7	3	3.6	9	12.7
	Post-	1,783	Nil	–	–	1[d]	–	3[d]	–
Totals during sampling periods									
Area 7						148	1,678	364	3,055
Fisher–Fitz Hugh						1,321	5,720	3,239	12,441
Bella Coola						402	1,037	622	1,608
Totals of pre- and post-sampling periods						49	1,318[f]	420	96,908[f]
Totals for 1964 catch						1,920	9,753	4,645	114,012

[a]Assumes same incidence of marks as during combined weeks 1–3 for ARV, and Bella Coola stream spawning population incidence for ALV.

[b]Estimated by extrapolating trend of incidence of marks from two adjacent sampling periods, assuming all pre- and post-season catches were taken during the previous or following week.

[c]Includes Dean Channel gillnet catches (11,951 total).

[d]Assumes same incidence of marks during pre-sampling period as in week 2, and same incidence of marks during post-sampling period as in week 6.

[e]Entirely Dean Channel.

[f]Variance estimated by assuming probable error ($p = 0.5$) of ±50% in estimates given above, footnotes a, b, and d.

number of marked fish included in the pre- and post-sampling catches and the probable error of the estimate.

Incidence of ARV and ALV marks in samples from the three fishing areas are presented in Fig. 3, 4, and 5. In each of these figures two shaded bands, the upper representing ALV, the lower ARV, depict the 50% confidence intervals of the incidence of marks in the samples obtained from spawned salmon in Bella Coola streams (these data reported in a later section). Wherever catches were largely from these stocks it is expected that sample mark incidence will fall close to these bands. Where the Bella Coola stock becomes diluted with fish from other stocks, the incidence of ARV is expected to fall. If the dilution was by the local Fisher Channel–Fitz Hugh stocks which were heavily marked, the incidence of ALV is expected to rise inversely to the fall of ARV. Where the dilution was from stocks not marked, then incidence of both ARV and ALV marked members is expected to fall.

Considering first the sample data obtained from area 7 catches (Fig. 3) it is apparent that the incidence of ARV marked fish in the first 3 weeks' catches was very close to that expected for the Bella Coola stock. The incidence fell sharply during the 4th week and fell to zero in the 6th week. The ARV incidence during the first 3 weeks indicates very little dilution by other stocks, and it would be expected that the ALV incidence would also reflect that of the Bella Coola stock. This is true for the samples obtained during weeks 1 and 3 but not for that of week 2. The week 2 sample, total of 4295 fish examined produced one ALV mark where four might have been the most probable occurrence. The probability of drawing one mark from a population where, using the same sample size, one would expect to draw four is about 0.07 (Poisson distribution), i.e. the result could have reasonably occurred as statistical error. It is also quite probable that a sampling mistake is involved, i.e. a fish marked ALV may have been missed in the sampling. Weighing all of the evidence together, it is suggested that the best estimate of the incidence of marks in the pre-sampling week catch would be an extrapolation of the Bella Coola spawning ground incidence of marks. This extrapolation implies that Bella Coola pink salmon formed a high precentage of the pre-sampling period catches. For both estimates a probable error of $\pm 50\%$ has been assigned. These estimates are shown graphically as hatched rectangles in Fig. 3, the black representing ALV and lying to the left of the ordinant, the white representing ARV and lying to the right of the ordinant.

From sample week 4 onward these data indicate that the Bella Coola stocks in fishing area 7 were progressively diluted by non-Bella Coola stocks. After week 4 the inverse relationship between the incidence of ARV and ALV suggests that local Fisher Channel–Fitz Hugh Sound stocks containing a high incidence of ALV marks became increasingly important components of the catch. To estimate the most probable incidence of ALV marks in the post-sampling catches, the trend line connecting sampling weeks 5 and 6 is extrapolated for a 1-week period. Again, a 50% confidence interval is indicated based on a probable error of $\pm 50\%$ in the estimate.

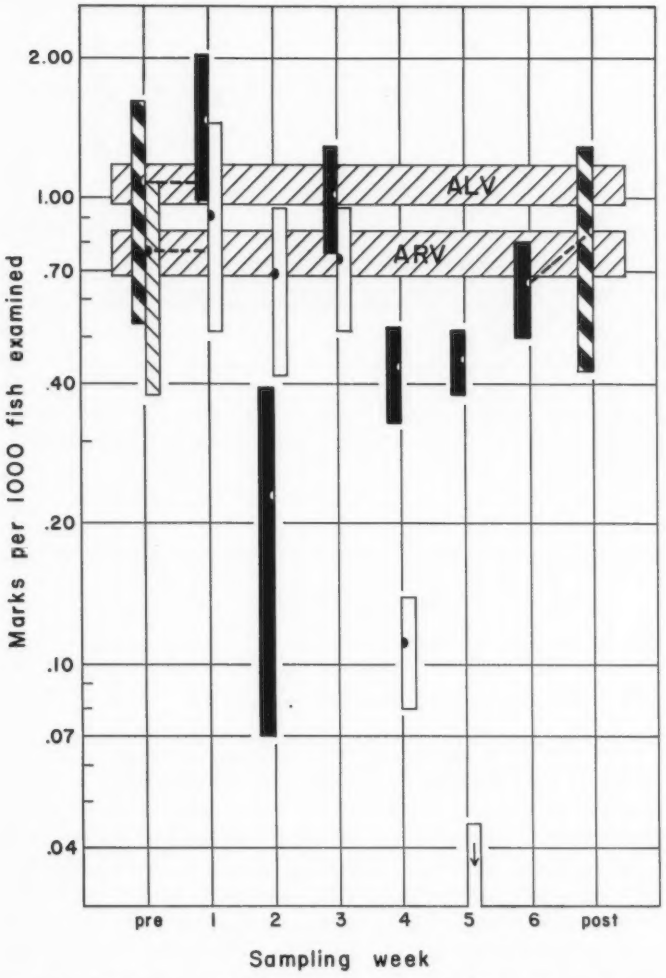

FIG. 3. Marks per 1000 fish sampled in area 7, 1964 catches. Columns depict estimate ±50% confidence interval. Black = ALV, white = ARV.

On the basis of these extrapolations, estimated numbers of ARV-and ALV-marked fish included in pre- and post-sampling catches in area 7 were computed. These estimates, totalling 8 ARV- and 76 ALV-marked fish, are presented in Table I.

Incidences of marked fish in the samples obtained from Fisher Channel–Fitz Hugh Sound catches are depicted in Fig. 4. The incidence of both marks appears to increase up to sampling week 3 from levels somewhat lower than

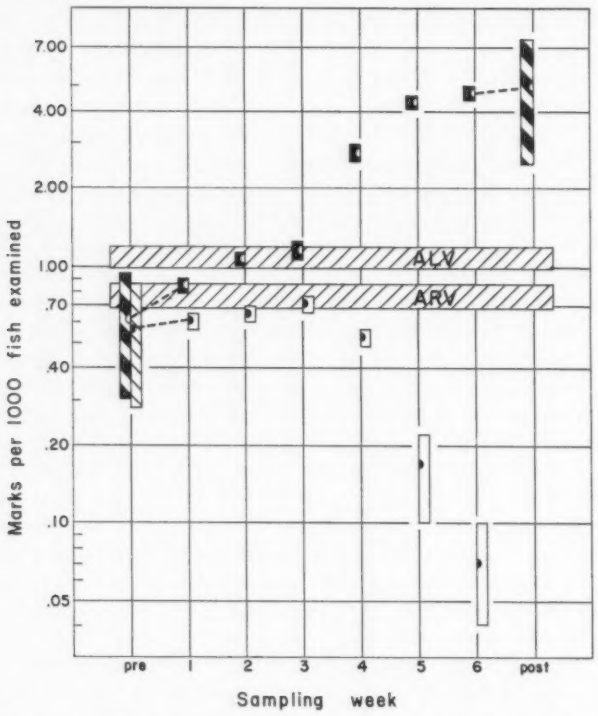

FIG. 4. Marks per 1000 fish sampled in Fisher Channel–Fitz Hugh Sound, 1964 catches. Columns depict estimate ±50% confidence interval. Black = ALV, white = ARV.

might be expected if the available fish had been entirely Bella Coola stock. The obvious trend in both lines suggests the presence of unmarked stocks early in the fishing season which gradually assumed a lesser relative importance or had left the area by week 3. Thus in estimating the incidence of both marks in pre-sampling period catches the trend line between weeks 1 and 2 have been extrapolated for a period of 1 week, ±50% probable errors are shown.

After week 3 the incidence of ARV marks fell to nearly zero indicating the passage of Bella Coola fish out of the fishing area. The incidence of ALV marks rapidly increased but the rate of increase slowed between sampling weeks 5 and 6. The trend line of week 5 to 6 has been extrapolated for a 1-week period to estimate the incidence of ALV marks in post-sampling catches and again probable error of ±50% has been indicated. Thus, in Table I, 36 ARV and 335 ALV marked fish have been estimated as contained in pre- and post-sampling catches from the Fisher Channel–Fitz Hugh Sound fishing areas.

In Fig. 5, incidences of marked fish in samples obtained from the Bella Coola gillnet fishery are similarly portrayed. Here the expected (or very nearly)

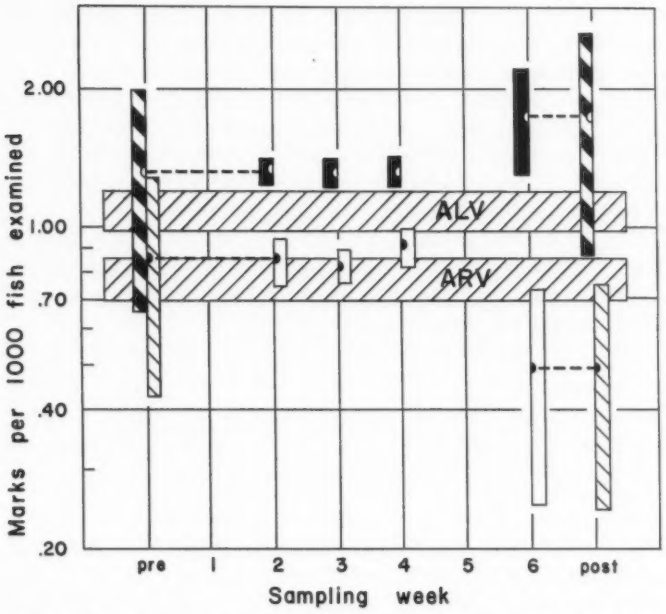

FIG. 5. Marks per 1000 fish sampled in Bella Coola gillnet area 1964 catches. Columns depict estimate ±50% confidence interval. Black = ALV, white = ARV.

incidence of marks for the Bella Coola spawning population was obtained from samples taken in weeks 2, 3, and 4. In weeks 1 and 5 fishing was not permitted in Burke Channel and North Bentinck Arm. A small catch was examined from Dean Channel in week 1. However, this sample cannot be used to indicate possible mark incidence in the pre-sampling period. The incidence of both marks is extrapolated from the average of samples obtained in sampling weeks 2, 3, and 4. A confidence interval of ±50% is shown. There is, in samples from weeks 2, 3, and 4, a tendency for the incidence of ALV marks to exceed that expected and for the ARV to be more nearly that expected from the spawning population sample. Although these differences are not statistically significant [$\chi^2_{ARV} = 1.518$, df = 4, $P > 0.8$ and $\chi^2_{ALV} = 4.710$, $P > 0.3$] they suggest the possible presence of a non-Bella Coola stock that had a high incidence of ALV marks. After the closure of week 5, the inverse relationship in the ARV: ALV ratio is evident suggesting an increased importance of the non-Bella Coola stock to the catch. The incidence of marks in week 6 samples were extrapolated for 1 week to estimate the post-season catch. A ±50% confidence interval is shown. These extrapolations lead to the estimates that 5 ARV and 9 ALV marked fish were taken in the pre- and post-sampling catches of the Bella Coola gillnet area, as shown in Table I.

In Table I the pre- and post-sampling catches of marked fish, for all areas, total 49 ARV and 420 ALV. In all cases probable error of ±50% has been assigned. This estimate leads to the estimation of the variances $\hat{\sigma}^2_{ARV} = 1318$ and $\hat{\sigma}^2_{ALV} = 96{,}908$. Combining these estimates and variances with those from sampling periods 1–6, the entire catch of marked fish is estimated at 1920 ARV with $\hat{\sigma}^2_{ARV} = 9753$ and 4645 ALV with $\hat{\sigma}^2_{ALV} = 114{,}012$. In the ALV estimate it is noted that the variance estimate is largely contributed from the post-sampling estimates.

Assumption 6.—That the variance estimates are sufficiently large to include the true reliability of the estimates of the catch of ARV and ALV marked fish. This assumption cannot be proven but is considered to be acceptable.

Spawning Escapement

Estimates of spawning escapements to streams which, because of their geographic locations, probably contributed members to either or both of the marked populations are listed in Table II. Of these streams, the Atnarko,

Table II. Estimated numbers of ARV and ALV marked pink salmon in area 8 escapements, 1964.

Spawning stream	Escapement		Sample (*pieces*)	Observed marks		Estimated total marked fish			
	No.	PE(%)		ARV	ALV	ARV	$\hat{\sigma}^2_{ARV}$	ALV	$\hat{\sigma}^2_{ALV}$
Atnarko	390,000	±10	47,481	41	43	337	5,263	353	5,634
Salloomt	2,500	±10	182	0	2	0		27	377
Snootli	12,000	±10	3,897	2	9	6	19	28	104
Necleetsconnay	14,000	±10	2,965	0	4	0		19	98
Kwatna	2,500	±20	719	0	1	0		3	10
Koeye	30,000	±20	287	0	7	0		732	121,798
Total						343	5,282	1,162	128,021

Salloomt, and Snootli are tributaries of the Bella Coola River. The Necleetsconnay and Bella Coola estuaries are adjacent in North Bentinck Arm. The Atnarko stock was undoubtedly the main source of the ARV marked population. The population of spawning pink salmon in the Snootli definitely contained ARV marked members. Some ARV fish may have entered the Salloomt. It appears that the Necleetsconnay population was not involved in the ARV marking.

Stocks of pink salmon endemic to the Kwatna and Koeye rivers, because of the geographic location of these streams, were not likely to have contributed members to the ARV population.

Spawning populations of North Bentinck Arm streams were repeatedly sampled and the total numbers in the escapements were estimated from counts made during the height of spawning activity. Indicated escapements are considered fairly accurate. Probable error (PE) of these estimates is estimated at $\pm 10\%$, based primarily on comparative tests made on Atnarko escapement the previous year (Parker, 1965). Indicated escapements to the Kwatna and Koeye rivers are not as reliable and PE is estimated at $\pm 20\%$.

These estimates and sample statistics lead to the estimated escapement of ARV = 343 with $\hat{\sigma}^2_{ARV} = 5282$ and ALV = 1162, $\hat{\sigma}^2_{ALV} = 128{,}021$. The major contribution to the variance of the ALV estimate arises from the Koeye River estimate, a combination of a large escapement and a small sample. Again it is considered that the variance estimates are sufficiently large to include the true reliability of the estimates of escapement of ARV and ALV marked pink salmon (Assumption 6).

An additional source of bias, possibly important, needs to be discussed. Estimates have been made on the original numbers of fish comprising two marked populations and the numbers of those populations returning to the catch and to the spawning escapement. Catch plus escapement is viewed as the total return and those fish which remain unaccounted for are presumed, or treated as, an estimate of fish dying from natural causes. There is a fourth category, however, comprised of those fish which return, encounter fishing gear which results in their death, but are not landed and therefore do not enter the catch. This loss may be important, especially in regard to the troll and gillnet gear. No direct observations are yet available. This problem is under study in Japan (Doi, 1962). Mortality from this source will bias the estimate of ocean mortality in the direction of being too great.

ESTIMATES OF MORTALITY

The various estimates of population parameters are summarized in Table III. With these estimates, natural mortality rates are computed using equations 1–3. Utilizing equation 3, survival of the ARV marked population during the 38-day period between mark releases is estimated as

$$\exp -m\ 38\ \bar{q}_c = \frac{2263}{5807} \cdot \frac{153{,}237}{166{,}862} = 0.358$$

with $\hat{\sigma}^2 = 13.01 \times 10^{-4}$.

Utilizing equation 2*a*, the total sea survival of the ARV marked group for approximately 450 days is

$$\exp -m(38\bar{q}_c + 412q_o) = \frac{2263}{166{,}862} = 0.0136$$

with variance $\hat{\sigma}^2 = 54.85 \times 10^{-8}$.

Adjusting these estimates to represent sums of the instantaneous natural mortality rates for *unmarked* fish, using $m = 1.38$, for the 38-day coastal period,

$$38\bar{q}_c = 0.744,\ \hat{\sigma}^2 = 53.30 \times 10^{-4}.$$

TABLE III. Estimates of sea survival rates of ARV and ALV marked Bella Coola pink salmon populations, 1962 brood-year.

	ARV	$\hat{\sigma}^2_{ARV}$	ALV	$\hat{\sigma}^2_{ALV}$
Released	166,862	696,688	153,237	266,116
Return:				
Fishery	1,920	9,753	4,645	114,012
Spawning escapement	343	5,282	1,162	128,021
Total	2,263	15,035	5,807	242,033
Survival	0.0136	54.85×10^{-8}	0.0379	10.32×10^{-6}

For the total sea period:

$$38\bar{q}_c + 412\bar{q}_o = 3.112, \quad \hat{\sigma}^2 = 15.57 \times 10^{-4}.$$

To obtain a result comparable among years, the coastal period is standardized as 40 days. Extrapolating the above result,

$$40\bar{q}_c = 0.783, \hat{\sigma}^2 = 59.06 \times 10^{-4}.$$

Subtracting the above value for 40 days from that for the total sea period,

$$410\bar{q}_o = 2.328, \hat{\sigma}^2 = 74.63 \times 10^{-4}$$

which estimates the sum of instantaneous natural mortality rates for the oceanic period.

The average instantaneous natural mortality rate per day for the coastal period is:

$$\bar{q}_c = 0.0196, \hat{\sigma}^2 = 3.691 \times 10^{-6}$$

and for the oceanic period:

$$\bar{q}_o = 0.0057, \hat{\sigma}^2 = 4.440 \times 10^{-8}.$$

The approximate 95% confidence interval, for the average coastal mortality rate $\bar{q}_c$, is $\underline{0.0158}$ to $\overline{0.0234}$ and for the average oceanic mortality rate $\bar{q}_o$, is $\underline{0.0053}$ to $\overline{0.0061}$. Thus the average day-to-day losses of the population to natural causes were most likely between 1.6 and 2.4% per day during the coastal period and between 0.5 and 0.6% during the oceanic period. The estimate for the oceanic period is biased by being too great. The average instantaneous natural mortality rate during the coastal period of sea life was 3–4 times greater than the average instantaneous natural mortality rate during the oceanic period.

1963 BROOD-YEAR

MARKED JUVENILES

Fish for the initial marking were seined in the eastern half of North Bentinck Arm, primarily between Whiskey Bay and Bella Coola (Fig. 1). Catches were transported to holding ponds alongside the laboratory anchored in Whiskey Bay. Seining commenced on April 20 and continued until April 24. Catches were held 24 hr or more before marking. Fish were brought into the laboratory, anaesthetized with 2-phenoxyethanol (Sehdev et al., 1963; Bell, 1964), marked by amputation of the adipose and right ventral fins (ARV) and, after recovery, transferred to a large holding pond (about 50 m^3).

During the marking the quality of marks produced was again sampled, leading to estimates of the numbers of ARV as well as various mismarks produced. The entire accumulated marked population was released at 1800 hr on April 26. It is estimated that 473,604 ARV marked pink salmon fry were released, with estimated variance, $\hat{\sigma}^2_{ARV} = 607{,}121$.

After the release of the ARV population daily samples were obtained from concentrations of fish found and examined for the presence of marked members. Again, the marked population rapidly spread out and mingled with unmarked fish. The apparent center of abundance of the marked population moved westward into Burke Channel and then southwestward in Burke Channel to its confluence with Fisher Channel and Fitz Hugh Sound.

As the marked population approached the lower reaches of Burke Channel the laboratory and fish-holding facilities were anchored in the entrance to Fougner Bay. Fish were again seined and transferred to the holding pens. These catches were exclusively obtained from the areas of Burke Channel east of Fougner Bay and west of Haaksvold Point. This restriction of catch area is believed to have effectively prevented the inclusion of Koeye River and other stocks originating in a number of small Fisher Channel and Fitz Hugh Sound streams. The inclusion to some degree of all Burke Channel and Bella Coola stocks was anticipated.

Marking commenced on May 26 and continued through May 28, following the routine specified for the earlier marking except that adipose and left ventral (ALV) fins were removed. During the preparatory anaesthesia, fish were graded according to size and only those of a size within the size distribution of the ARV recaptures were marked. This tactic was to insure that the natural mortality rate estimate was applicable to a specified life-history period, i.e. for a 33-day period commencing within a day or two of first entry into the sea.

It is estimated that 155,824, with $\hat{\sigma}^2 = 80{,}409$ ALV-marked pink salmon were released from the second marking.

Problems arising from mismarking were not serious and again will be met simply by dealing only with fish marked ARV and ALV in the adult return. It is probable that some of the ARV marks were altered into ABV by chalimus larvae of *Caligus clemensi*. Bias from this source is again assumed

(Assumption 4) to be too small to significantly affect the mortality estimates. Alteration of ALV to ABV is not considered likely because of the advanced size of the ALV-marked fish.

After the release of the ALV population both marks were observed in catches of fish obtained up to June 15, at which time sampling of the juvenile population was terminated. Sampling was conducted both with seines and with a 2-boat surface trawl (Tyler and Bevan, 1964). The marked populations were fairly well mixed together with the unmarked populations. As revealed by the trawl samples, the marked populations turned north into Fisher Channel and largely remained in that area when sampling was discontinued. Some portions of the population moved into Johnson and Return channels and into Lama Pass, presumably migrating to the open sea through these northern routes. Observations in Nalau Pass indicated that relatively small numbers of the population used that entrance into Queen Charlotte Sound.

1965 ADULT RETURN

Commercial Fishery Catch

Commercial catches of 1963 brood-year pink salmon in 1965 from areas 7 and 8 are summarized in Table IV. As in the treatment for the 1962 brood-year, the catches have been apportioned within area 8 among the Fisher Channel–Fitz Hugh Sound area and the Bella Coola gillnet area. Troll catches are included in the Fisher Channel–Fitz Hugh Sound catch and in the area of catch. These were, in each case, about 1.8% of the total catch.

From sample statistics the number of ARV- and ALV- marked pink salmon landed in the catches are estimated with their variances. Pre- and post-sampling catches of marked fish are again estimated by extrapolation of the sample values of mark incidence to a 1-week pre- and post-sampling period. Details are given in Table IV and the extrapolations are shown in Fig. 6 and 7.

A single sample was obtained from area 6 fisheries in Laredo Sound, week 1, indicating that the marked population was involved in catches from that area (1320 examined, 1 ARV, 1 ALV). There is no additional information. However, it can again be stated that members of the marked population were taken by area 6 fisheries and will be included in the "ocean" mortality estimate. Samples obtained from area 9 catches during week 5 (2530 examined, 2 ARV) indicate a bias of similar direction but, because of the small and sporadic nature of the fishery, less severe.

The total estimates of the area 7 and 8 catch of marked pink salmon are 1517 ARV, $\hat{\sigma}^2_{ARV} = 4886$ and 1426 ALV, $\hat{\sigma}^2_{ALV} = 7732$. About half of the variance associated with the ALV estimate arises from the estimates of the post-sampling week catches.

Spawning Escapements

Estimates of spawning escapements to Burke Channel and North Bentinck Arm streams are presented in Table V. All are based on crude census methods:

TABLE IV. Estimated numbers of ARV and ALV marked pink salmon taken by areas 7 and 8 fisheries in 1965.

		Catch (*pieces*)	Sample (*pieces*)	Observed		Estimated			
				ARV	ALV	ARV	$\hat{\sigma}^2_{ARV}$	ALV	$\hat{\sigma}^2_{ALV}$
Area 7									
	Pre-	5,816	Nil	–	–	0[a]	–	3[a]	–
July 19–23	1	32,093	11,240	1	6	3	8.2	17	48.9
July 26–30	2	117,799	68,544	13	15	22	38.4	26	44.3
Aug. 2–6	3	203,322	57,438	50	42	177	626.5	149	526.3
Aug. 9–13	4	139,313	39,087	52	35	185	660.6	125	444.6
Aug. 16–20	5	67,277	21,130	28	24	89	283.8	76	243.3
Aug. 23–27	6	23,101	10,443	1	26	2	4.9	58	127.2
	Post-	7,962	Nil	–	–	0[b]	–	30[b]	–
Fisher–Fitz Hugh									
	Pre-	6,130	Nil	–	–	10[a]	–	11[a]	–
	1	29,957	5,053	8	9	47	281.2	53	316.3
	2	59,817	17,996	19	15	63	209.9	50	165.7
	3	147,422	51,243	129	90	371	1,067.7	259	744.9
	4	173,841	49,171	93	74	329	1,162.4	262	924.9
	5	32,847	15,170	22	31	48	103.2	67	145.3
	6	20,873	6,907	9	51	27	82.2	154	465.8
	Post-	2,738	Nil	–	–	3[b]	–	35[b]	–
Bella Coola[c]									
	Pre-	208	Nil	–	–	0[d]	–	0[d]	–
	1	116[e]	116	1	–	1	1.0	–	0
	2	14,713	13,829	25	7	27	28.3	7	7.9
	3	53,224	25,472	54	21	113	235.8	44	91.7
	4	Closed	–	–	–	–	–	–	–
	5	Closed	–	–	–	–	–	–	–
	6	Closed	–	–	–	–	–	–	–
	Post-	328	Nil	–	–	0[d]	–	0[d]	–
Totals during sampling periods									
Area 7						478	1,622	451	1,435
Fisher–Fitz Hugh						885	2,907	845	2,763
Bella Coola						141	265	51	100
Totals of pre- and post-sampling periods						13	92[f]	79	3,434[f]
Totals for 1965 catch						1,517	4,886	1,426	7,732

[a]Assumes same mark incidence as in week 1.
[b]Estimated by extrapolating trend of incidence of marks from two adjacent sampling periods, assuming all pre- and post-season catches were taken during the previous or following week.
[c]Includes Dean Channel gillnets catches.
[d]Assumes no marks were taken in these small catches.
[e]Entirely Dean Channel.
[f]Variance estimated by assuming probable error ($p = 0.5$) of $\pm 50\%$ in estimates given.

counts and estimates made during the peak of the spawning period. In addition, the spawning populations of the Atnarko and Salloomt Rivers were estimated by a tag and recovery technique (Seber–Jolly method). Details of this estimation are given in the Appendix. The tag and recovery estimate is known to

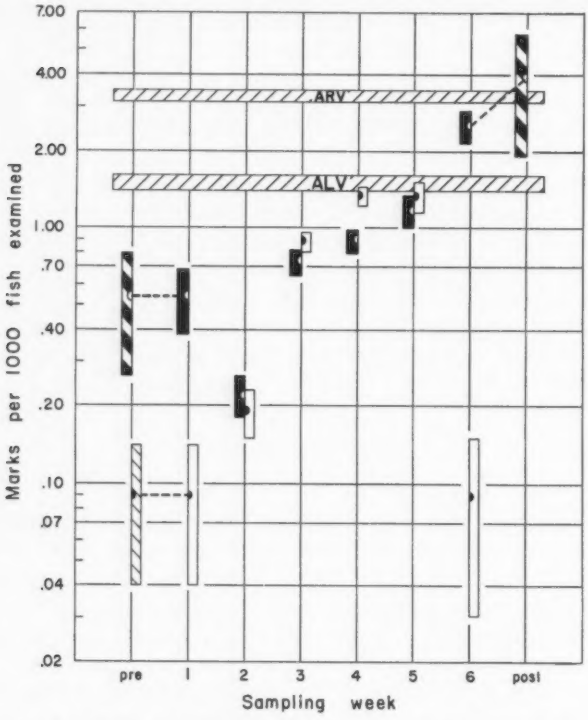

FIG. 6. Marks per 1000 fish sampled in area 7, 1965 catches. Columns depict estimate ±50% confidence interval. Black = ALV, white = ARV.

underestimate, for it estimated only that part of the spawning population which became available to sampling after death. Losses to the population of carcasses occurred from bears, drift out of the study area, log jams, and sanding over in deep still areas. The estimate from tag and recovery on the Atnarko River was 114,532. This is 23,583 or 17% less than that derived from the count-estimate method. Because of the known bias, it was concluded that the crude census estimate of 150,000, PE ±10%, is quite realistic.

The spawning population in the Salloomt River was estimated by the Seber–Jolly method to be 1413 fish. In this small stream it is expected that very little loss to availability would occur from drift-out or sanding. Bear predation, however, was very heavy and with a decided selectivity for males. The resulting sex ratio of the total estimate was 1.26 ♂: 8.74 ♀. The estimated sex ratio on the Atnarko was 3.54 ♂: 6.46 ♀. If it is assumed that the Atnatko escapement sex ratio reflects that of the parent population escaping to the Salloomt, by direct proportion an adjusting coefficient of 3.7 is indicated to correct the effect of predation on the sex ratio. Since bear predation of live

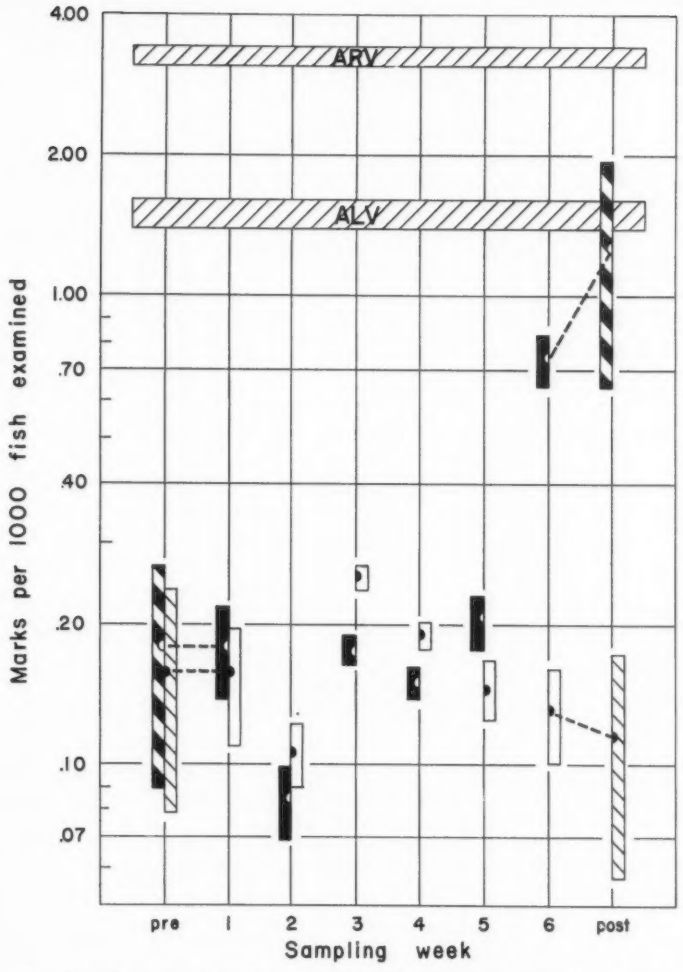

FIG. 7. Marks per 1000 fish sampled in Fisher Channel–Fitz Hugh Sound 1965 catches. Columns depict estimate ±50% confidence interval. Black = ALV, white = ARV.

fish was not exclusively on the male component, this can only be considered as an incomplete correction. These data and adjustments lead to the estimate of the Salloomt spawning escapement of 1861. Since this is still considered as an underestimate, a total population of 2000, PE ±10%, appears realistic.

Less information is available for the remaining streams and higher probable errors have been arbitrarily assigned. The resulting variance estimates are deliberately generous. It is, therefore, with a high degree of confidence that

TABLE V. Estimated escapement of marked pink salmon in Burke Channel and North Bentinck Arm streams, 1965.

	Estimated total escapements			Sample statistics					Estimate of total marks			
Stream	No. of fish	$\overline{PE}^a$ (%)	$\hat{\sigma}^2$	No. examined	ARV	$\hat{\sigma}^2$	ALV	$\hat{\sigma}^2$	ARV	$\hat{\sigma}^2_{ARV}$	ALV	$\hat{\sigma}^2_{ALV}$
Atnarko	150,000	±10	4.945×10^5	69,936	229	229	105	105	491	6,349	225	1,594
Salloomt	2,000	±10	8.792×10^4	829	2	2	2	2	5	13.05	5	13.05
Necleetsconnay	1,500	±20	1.976×10^5	296	1	1	1	1	5	27.20	5	27.20
Nooseseck	3,000	±20	7.913×10^5	737	2	2	0	0	8	37.63	0	0
Kwatna	20,000	±25	5.495×10^7	454	0	0	4	4	0	0	176	11,922
Total									509	6,427	411	13,556

[a]This estimate, analogous to a 50% confidence interval, is largely subjective, but based on whatever clues are available to indicate a range of values considered as real and likely possibilities. The estimates of σ^2 are derived from these values as: $\left[\frac{PE}{0.6745}\right]^2$.

the escapement portion of the ARV-marked population is estimated at 509, $\hat{\sigma}^2_{ARV} = 6427$ and of the ALV-marked population is estimated at 411, $\hat{\sigma}^2_{ALV} = 13{,}556$.

ESTIMATES OF MORTALITY

The various estimates of population parameters and their variances are summarized in Table VI. Utilizing equation 3, survival of the ARV-marked population during the 33-day period between the two mark releases is estimated as

$$\exp -m33\bar{q}_c = \frac{2026}{1837} \cdot \frac{155{,}824}{473{,}604} = 0.363$$

with $\hat{\sigma}^2 = 11.95 \times 10^{-4}$.

TABLE VI. Estimates of sea survival rates of ARV and ALV marked Bella Coola pink salmon populations, 1963 brood-year.

	ARV	$\hat{\sigma}^2_{ARV}$	ALV	$\hat{\sigma}^2_{ALV}$
Released	473,604	706,121	155,824	80,409
Return:				
Fishery	1,517	4,886	1,426	7,732
Spawning escapement	509	6,427	411	13,556
Total	2,026	11,313	1,837	21,288
Survival	0.0043	5.101×10^{-8}	0.0118	8.785×10^{-7}

Utilizing equation 1 the total sea survival from natural deaths of the ARV population is, for the approximately 450-day period:

$$\exp -m(33\bar{q}_c + 417\bar{q}_o) = \frac{2026}{473{,}604} = 0.0043$$

with $\hat{\sigma}^2 = 5.101 \times 10^{-8}$. Again Assumption 3 is employed, $S_{Ma} = 1$ and the estimate is biased in the direction of being too large because of the unknown losses to area 6 and 9 fisheries and "drop-out" mortality.

Assuming the long-term marking mortality coefficient $m = 1.38$, for the initial 33-day coastal period

$$33\bar{q}_c = 1.011/1.38 = 0.733; \hat{\sigma}^2 = 4.763 \times 10^{-3}.$$

The average instantaneous natural mortality rate on a per day basis is $\bar{q}_c = 0.0222$, $\hat{\sigma}^2 = 4.374 \times 10^{-6}$. Extrapolating this estimate to include 40 days at the same average rate

$$40\bar{q}_c = 0.888, \hat{\sigma}^2 = 6.997 \times 10^{-3}.$$

Similarly,

$$33\bar{q}_c + 417\bar{q}_o = 5.449/1.38 = 3.949;\ \hat{\sigma}^2 = 1.449 \times 10^{-3}.$$

By subtraction, the natural mortality rate for the oceanic period is estimated as

$$410\bar{q}_o = 3.060,\ \hat{\sigma}^2 = 8.446 \times 10^{-3}.$$

The average instantaneous natural mortality rate on a per day basis is $\bar{q}_o = 0.0075$, $\hat{\sigma}^2 = 5.024 \times 10^{-8}$. The approximate 95% confidence interval for $\bar{q}_c$ is $\underline{0.0181}$ to $\overline{0.0263}$ and for $\bar{q}_o$ is $\underline{0.0071}$ to $\overline{0.0079}$.

Again, particularly since the estimate of $\bar{q}_o$ is biased in the direction of being too great, there is little doubt but that these results indicate significant differences between the average daily mortality rates in the coastal and oceanic phases of 1963 brood-year pink salmon. This conclusion further supports the general hypothesis being tested.

DISCUSSION

There can be little doubt that the day-to-day relative losses of pink salmon in the marine environment are usually much higher in the initial coastal period than in the later oceanic period. This conclusion has been consistent from the series of tests conducted on the 1961, 1962, and 1963 brood-years. It is now possible to state that during the initial 40-day period of sea life *average* daily relative loss to the population was about 2–4% and during the following 410-day period *average* daily loss was about 0.4–0.8%. The latter is certainly a maximum estimate because of bias introduced by catches of unknown magnitude and unreported deaths associated with fishing gear.

Although these conclusions have narrowed the problem of sea mortality, they fall far short of the goal of understanding it. For example, there is no magical transition from high to low mortality rate at 40 days. To the best of our knowledge the fish remain in the coastal waters for at least an additional 40 days, perhaps longer. It would seem prudent to anticipate that mortality, rate would remain high, and consequently the true oceanic mortality rate would be much less than indicated by the above averages. Furthermore, the distribution of mortality within the first 40 days is not known. Even within this short time-period the average rates may be misleading.

Finally, the variability among the mortality estimates must be recognized. Among the 3 brood-years deviations from the average in both coastal and oceanic estimates have been ±36–38%. Although some of this variability is undoubtedly due to inherent statistical error, it would seem unwise to consider any portion of natural mortality in the sea as a constant. This conclusion has far-reaching implications to the prospects of predicting returning runs of pink salmon from the numbers of fry migrating to the sea. Furthermore, natural sea mortality can hardly be classed as compensatory, therefore the variation

serves to obscure a parental-to-filial generation relationship currently being sought for in stock and recruitment curves.

To illustrate the influence of variability of natural mortality rates in the sea the available estimates have been applied to stock parameters for brood-years 1960–63 and stock sizes at various stages have been computed as shown in Table VII. The total sea survival for the 1960 brood-year was calculated

TABLE VII. Comparison of stock size and levels of mortality among 1960–63 brood-years, Bella Coola pink salmon.

Brood-year:	1960[a]	1961	1962	1963
Return:	1962	1963	1964	1965
No. of fry entering the sea	73,400,000	55,700,000	61,200,000	32,100,000
Per cent surviving at 40 days		23.4	45.1	41.1
No. surviving at 40 days		13,000,000	27,600,000	13,200,000
Per cent surviving "oceanic"		22.2	9.8	4.7
Per cent surviving total sea	21.8	5.2	4.4	1.9
No. returning to coast	16,000,000	2,900,000	2,700,000	620,000
Per cent caught by fishery[b]	75.0	84.6	85.2	74.9
Catch	12,000,000	2,500,000	2,300,000	460,000
Escapement	4,000,000	400,000	400,000	160,000
Per cent return taken in area 7		9.2	6.2	23.6

[a]Based on Hook Nose Creek survival data, catch and escapements are very rough approximations.

[b]Includes catches from areas 7 and 8 only.

from the Hook Nose Creek stock (Parker, 1964) and is applied to a catch of approximately 12,000,000 and an escapement of about 4,000,000. These estimates and the use of Hook Nose Creek data must be considered as rough approximations only. Nevertheless the implications of natural sea mortality on the relative success or failure of a run are clear.

These implications underline both the importance of sea mortality and the present areas of ignorance concerning the scientific management of pink salmon. The technique used in this study is severely limited by the availability of only two comparable fin marks. The sources of mortality are little understood, whether due to natural agents or fishing. It would seem imperative to now pursue the study of marine mortality of pink salmon from an ecological basis to gain an understanding of the agencies of loss and the possibility of enhancing the yield by manipulation of the biological relationships.

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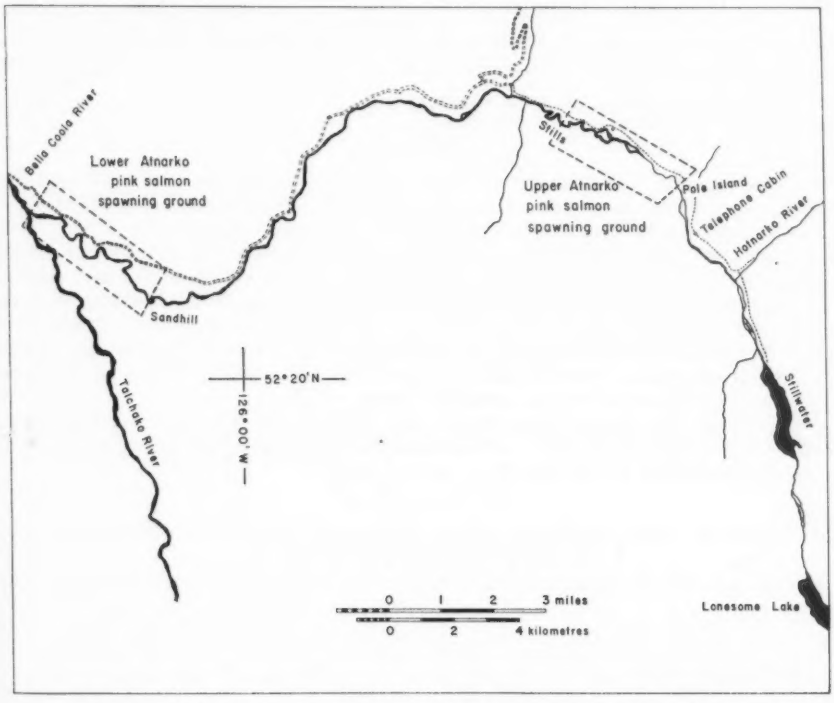
(Appendix *follows*)

APPENDIX

ATNARKO RIVER SPAWNING GROUND ESTIMATES

The main contribution to area 8 stocks of pink salmon is that of the Atnarko River (App. Fig. 1) and major effort was directed to the enumeration of that population. The Atnarko is a clear-water stream arising in the high plateau country east of the coastal mountains. About 35 miles from the sea the Atnarko joins the glacial Talchako River and together they form the Bella Coola River (Fig. 1). Except in the winter, the Bella Coola is muddy. However, when the pink salmon are moving upstream there are a few areas in which they accumulate and may be observed surfacing.



APP. FIG. 1. Pink salmon spawning areas of the Atnarko River, British Columbia.

During September frequent observations were made to determine the time when there were few fish left in the main Bella Coola River. During the same period, frequent observations were made on the progress of the spawning population in the Atnarko River. By September 15 fish could no longer be seen surfacing in the Bella Coola. Accumulations in resting places in the Atnarko were diminishing and spawning activity was general. On September 18, 19, and 20 a crude census was taken on the basis of counts and estimates made from a rubber raft and from the banks of the Atnarko from Stillwater to the Talchako confluence. These estimates are given by stream sections in App. Table I. The conservation officer at Bella Coola later estimated that approximately 3000 pink salmon spawned in the Atnarko above Stillwater. On this basis it is estimated that about 150,000 pink salmon formed the Atnarko River escapement.

APP. TABLE I. Estimates of spawning population of Atnarko River pink salmon made by counts and estimates, September 18–20, 1965.

Date	River section	Live	Dead (15%)	Total
Sept. 18	Stillwater to Hotnarko	2,000	300	2,300
" 18	Hornarko to telephone cabin	4,500	675	5,175
" 18	Telephone cabin to Pole Island	2,000	300	2,300
" 19	Pole Island to Stills[a]	57,500	8,625	66,125
" 19	Stills to Sandhill	2,400	360	2,760
" 20	Sandhill to Talchako[b]	62,600	9,390	71,990
	Totals	131,000	19,650	150,650

[a]Section designated as upper Atnarko
[b]Section designated as lower Atnarko.

The foregoing method of enumerating a spawning population has the severe limitation of being largely subjective. A second method was used to estimate the spawning population to serve as a check on the first. This method is based on techniques proposed by Seber (1962, 1965) and Jolly (1963, 1965) and adapted to the problem of estimating spawning populations of Pacific salmon by Dr G. J. Paulik (personal communication). Whereas Dr Paulik also gave methods for variance estimates based on Jolly (1965), I have used the methods for combining variances given by Deming (1943), as in the text. Only fish which have died are involved. At sequential times (not necessarily equal intervals) the spawning grounds are searched for dead fish. As each dead fish is encountered, it is either tagged and returned to the place where it is found or removed from the stream were it will not be encountered again.

The model and notation is presented in App. Table II. This schema is taken from that used by Mr R. Worlund (personal communication) on applications of the method to estimating spawning populations of Columbia River chinook salmon.

APP. TABLE II. Model and notation used in application of Seber–Jolly method to estimating spawning ground populations.

Time	t_0	t_1	t_2	t_3	t_4	t_5
No. of fish dying		D_0	D_1	D_2	D_3	D_4
Proportion "surviving"		S_0	S_1	S_2	S_3	S_4
No. of D_i available		B_0	B_1	B_2	B_3	B_4
Total no. of available carcasses (tagged and untagged)		N_1	N_2	N_3	N_4	N_5
Total carcasses recovered		C_1	C_2	C_3	C_4	C_5
No. of tags released		T_1	T_2	T_3	T_4	T_5
No. tags recovered in C_i		$R_{1\cdot}$	$R_{2\cdot}$	$R_{3\cdot}$	$R_{4\cdot}$	$R_{5\cdot}$
No. tags recovered from T_i		$R_{\cdot 1}$	$R_{\cdot 2}$	$R_{\cdot 3}$	$R_{\cdot 4}$	$R_{\cdot 5}$
No. tags released before ith sample and recovered after ith sample		Ξ_1	Ξ_2	Ξ_3	Ξ_4	Ξ_5
No. of tags available		M_1	M_2	M_3	M_4	M_5
Probability of recovering a carcass		u_1	u_2	u_3	u_4	u_5
Proportion of population of carcasses that are tagged		f_1	f_2	f_3	f_4	f_5

The model implies that, after spawning, fish die and *become available for sampling* however temporary "availability" is. During the first period (t_0 to t_1) a number of fish die (D_0) and become available. At t_1 not all remain available, the fraction which does is $(S_0)^{1/2}$, i.e. "survival" is estimated for one-half the total time period. Thus $D_0(S_0)^{1/2} = B_0 = N_1$. Of this number only a fraction (u_1) are encountered and examined, i.e., $B_0u_1 = N_1u_1 = C_1$. Of the fraction not encountered $B_0(1-u_1)$, only S_1 remain available until t_2. Also available at t_2 is $D_1(S_1)^{1/2} = B_1$ so that the total number of available carcasses at t_2, $N_2 = B_0(1-u_1)S_1 + B_1$ and $C_2 = N_2u_2$. The various parameters are estimated from the statistics on the recovery of marked fish (T_i) replaced in the population of available but unencountered fish. The main underlying assumptions are (1) the population of tagged carcasses is representative of the population of untagged carcasses, an assumption inherent in all such estimation techniques; and (2) that a carcass once removed from the available population does not return.

The estimating equations are as follows.

The total spawning population that becomes available (temporarily or otherwise) E is estimated by

$$\hat{E} = \sum_{t=0}^{t=I-1} \hat{D}_i \qquad \ldots\ldots(1a)$$

where I denotes the last day of sampling.

$$\hat{\sigma}^2_E = \sum_{t=0}^{t=I-1} \hat{\sigma}^2_{D_i} \qquad \ldots\ldots(1b)$$

$$\hat{M}_i = \frac{T_i \Xi_i}{R_{\cdot i}} + R_i. \qquad \ldots\ldots(2a).$$

The variances of the numbers of tags recovered in the samples examined are estimated by $\hat{\sigma}^2 = nPQ$ and summed to obtain

$$\hat{\sigma}^2_{\Xi_i}, \hat{\sigma}^2_{R\cdot i}, \text{ and } \hat{\sigma}^2_{Ri\cdot},$$

$$\hat{\sigma}^2_{M_i} = \left(\frac{T_i \Xi_i}{R_{\cdot i}}\right)\left[\left(\frac{\hat{\sigma}_{\Xi_i}}{\Xi_i}\right)^2 + \left(\frac{\hat{\sigma}_{R\cdot i}}{R_{\cdot i}}\right)^2\right] + \hat{\sigma}^2_{Ri\cdot} \qquad \ldots\ldots(2b)$$

$$\hat{f}_i = R_{i\cdot}/C_i \qquad \ldots\ldots(3a)$$

$$\left(\frac{\hat{\sigma}_{f_i}}{\hat{f}_i}\right)^2 = \left(\frac{\hat{\sigma}_{Ri\cdot}}{R_{i\cdot}}\right)^2 \qquad \ldots\ldots(3b)$$

$$\hat{N}_i = \hat{M}_i/\hat{f}_i \qquad \ldots\ldots(4a)$$

$$\hat{\sigma}^2_{N_i} = \hat{N}_i^2\left[\left(\frac{\hat{\sigma}_{M_i}}{\hat{M}_i}\right)^2 + \left(\frac{\hat{\sigma}_{f_i}}{\hat{f}_i}\right)^2\right] \qquad \ldots\ldots(4b)$$

$$\hat{S}_i = \frac{\hat{M}_{i+1}}{\hat{M}_i - R_{i\cdot} + T_i} \qquad \ldots\ldots(5a)$$

$$\hat{\sigma}^2_{S_i} = \hat{S}_i^2\left[\left(\frac{\hat{\sigma}_{Mi+1}}{\hat{M}_{i+1}}\right)^2 + \frac{\hat{\sigma}^2_{Mi} + \hat{\sigma}^2_{Ri}}{(\hat{M}_i - R_i + T_i)^2}\right] \quad \ldots\ldots(5b)$$

$$\hat{u}_i = C_i/\hat{N}_i \quad \ldots\ldots(6a)$$

$$\hat{\sigma}^2_{u_i} = u_i^2\left(\frac{\hat{\sigma}_{Ni}}{\hat{N}_i}\right)^2 \quad \ldots\ldots(6b)$$

$$\hat{B}_i = \hat{N}_{i+1} - \hat{S}_i(\hat{N}_i - C_i + T_i) \quad \ldots\ldots(7a)$$

$$\hat{\sigma}^2_{Bi} = \hat{\sigma}^2_{Ni+1} + \hat{\sigma}^2_{Si}(\hat{N}_i - C_i + T_i)^2 + \hat{\sigma}^2_{Ni}\hat{S}_i^2 \quad \ldots\ldots(7b)$$

$$\hat{D}_i = \hat{B}_i/\hat{S}_i^{\frac{1}{2}} \quad \ldots\ldots(8a)$$

$$\hat{\sigma}^2_{Di} = \hat{D}_i^2\left[\left(\frac{\hat{\sigma}_{Bi}}{\hat{B}_i}\right)^2 + \left(\frac{\hat{\sigma}_{Si}}{2\hat{S}_i}\right)^2\right] \quad \ldots\ldots(8b).$$

D_0, D_1, and D_{I-1} cannot be calculated directly. It is assumed that $u_1 = u_2$. Then

$$\hat{N}_1 = C_1/\hat{u}_2 \quad \ldots\ldots(9a)$$

which with equations 7 and 8 yields D_1. Since u is related to the effort expended upon recovery of carcasses it is under the control of the investigator and the assumption that $u_1 = u_2$ can be made acceptable. The efficiency of effort, however, is subject to change both by physical conditions and by previous learning experience. In order to estimate the variance of N_1 I have doubled $\hat{\sigma}_{u_2}$, thus

$$\hat{\sigma}^2_{N1} = \hat{N}_1^2\left(\frac{2\hat{\sigma}_{u2}}{\hat{u}_2}\right)^2 \quad \ldots\ldots(9b).$$

It is noted that $B_0 = N_1$ and if one may assume a value for S_0, then where the time period t_0 (the start of the die off) to t_1 is equal to t_1 to t_2,

$$\hat{D}_0 = \hat{N}_1/\sqrt{\hat{S}_0} \quad \ldots\ldots(10).$$

If the time periods are unequal, then

$$\hat{D}_0 = \hat{N}_1 \exp\left(-\frac{t_1 - t_0}{2\,(t_2 - t_1)}\, ln\, \hat{S}_0\right) \quad \ldots\ldots(10\text{ alt.}).$$

Survival (S) is related to the rate at which fish disappear from the stream sites inspected and, therefore, are not under the control of the investigator but the result of flow conditions and removal by animals feeding upon the available carcasses. In the present context, water levels were steady during the period t_0 to t_2 and $S_0 = S_1$ might therefore be considered. On the other hand, the number

of fish dying was small, and the fraction removed by a constant bear-and-bird population would diminish with increase in the number dying. A further factor might be an increased rate of disappearance from sanding over as spawning activity increased. Thus it is suspected that $S_0 < S_1$ in this particular case. I have *subjectively* assumed that S_0 had a value between 0.20 and 1.00, used the midpoint 0.6, and set the range equal to $\pm 2\hat{\sigma}_{S_0}$. Thus $\hat{S}_0 = .6$, $\hat{\sigma}_{S_0}^2 = .04$ appears to encompass the range of likely possibilities and provide an acceptable error term.

D_{I-1} is estimated as follows:

$$(\hat{S}_{I-1}\ \hat{u}_I) = \frac{R_{I\cdot}}{(\hat{M}_{I-1} - R_{I-1\cdot} + T_{I-1})} \qquad \ldots\ldots(11).$$

Then

$$\hat{S}_{I-1} = \frac{(\hat{S}_{I-1}\ \hat{u}_I)}{\hat{u}_{I-1}} \qquad \ldots\ldots(12a)$$

where u_I is assumed equal to u_{I-1}, and where $\hat{\sigma}_{u_I} = 2\hat{\sigma}_{u_{I-1}}$ (as discussed above).

$$\left(\frac{\hat{\sigma}_{S_{I-1}}}{\hat{S}_{I-1}}\right)^2 = 4\left(\frac{\hat{\sigma}_{u_{I-1}}}{\hat{u}_{I-1}}\right)^2 + \left(\frac{\hat{\sigma}_{R_{I\cdot}}}{R_{I\cdot}}\right)^2 + \frac{\hat{\sigma}_{M_{I-1}}^2 + \hat{\sigma}_{R_{I-1\cdot}}^2}{(\hat{M}_{I-1} - R_{I-1\cdot} + T_{I-1})^2} \qquad \ldots\ldots(12b)$$

$$\hat{M}_I = (\hat{M}_{I-1} - R_{I-1\cdot} + T_{I-1})\,\hat{S}_{I-1} \qquad \ldots\ldots(13a)$$

and

$$\left(\frac{\hat{\sigma}_{M_I}}{\hat{M}_I}\right)^2 = \left(\frac{\hat{\sigma}_{S_{I-1}}}{\hat{S}_{I-1}}\right)^2 + \frac{\hat{\sigma}_{M_{I-1}}^2 + \hat{\sigma}_{R_{I-1\cdot}}^2}{(\hat{M}_{I-1} - R_{I-1\cdot} + T_{I-1})^2} \qquad \ldots\ldots(13b)$$

and with equations 4, 7, and 8 D_{I-1} is estimated.

One must have knowledge of the time when spawning fish first die (t_0). There must be at least 5 tagging and sampling days during the die-off period. All recovered carcasses must be either tagged and returned to their original position or removed from the population. Some fraction of the spawning population never becomes available to sampling. Those fish which drift out of the area or into unavailable locations without first becoming available, or are removed by predators although yet alive, are not estimated by this treatment. Thus, to the degree that fish are never available, the Seber–Jolly method underestimates the spawning population.

In applying this Seber–Jolly method to the problem of estimating the Atnarko spawning pink salmon population, the two main spawning sections, designated as "upper" and "lower" Atnarko were studied as discrete units. Furthermore, the sexes were treated separately in each section as a possible means of detecting errors in the estimates (the sum of the estimates for each sex was very close to the estimate for sexes combined). Tagging was accomplished by slipping a coloured rubber band over the caudal peduncle of the fish left in situ.

Appendix Table III presents the data and the progression of steps utilizing equations 1–13 for the upper Atnarko River. Appendix Table IV presents the equivalent data and treatment for the lower Atnarko River.

Difficulty was experienced in obtaining sufficient rubber bands, and from September 25–27 it was attempted to remove about 75% of the dead fish from the available population. No fish were tagged during this period and the recovery data are not used in the calculations because of the impossibility of calculating Ξ_3. It is noted that S_2, the calculated survival from t_2 to t_3 is very low in both cases.

APP. TABLE III. Estimation of the spawning population of upper Atnarko pink salmon in 1965, Seber–Jolly method.

(a) Time periods, tagging, and recovery data

Tagging			Recovery period[a]						
Period	Date	No.	2	3[b]	4	5	6	7	$R_{\cdot i}$
1	Sept. 17	967	191	(137)	21	8	4	1	225
			184.0		20.9	8.0	4.0	1.0	217.9
2	Sept. 23	5066		(2621)	523	154	41	10	728
3	Sept. 26–27	0			487.7	149.6	40.0	9.7	687.0
4	Oct. 1	1842				1049	146	6	1201
						842.6	133.1	5.9	981.6
5	Oct. 4	814					252	17	269
							213.6	16.2	229.8
6	Oct. 7	288						52	52
								44.6	44.6
7	Oct. 10	0							
$R_{i\cdot}$			191		544	1211	443	86	
			184.0		508.6	1000.2	390.7	80.9	

(b) Estimate of population

i	T_i	Ξ_i	$R_{i\cdot}$	$R_{\cdot i}$	C_i	$\hat{M}_i$	$\hat{f}_i$	$\hat{N}_i$	$\hat{S}_i$	$\hat{u}_i$	$\hat{B}_i$	$\hat{D}_i$
0									0.6			2,795
1	967	0		225	967			2,165	0.4422		10,694	16,081
2	5,066	34	191	728	5,205	427.6	0.0367	11,651	0.1656	0.4467	10,606	26,065
3	0				(12,069)							
4	1,842	218	544	1,201	7,745	878.4	0.0702	12,512	0.8456	0.6190	2,516	2,736
5	814	208	1,211	269	5,332	1,840.4	0.2271	8,104	0.4374	0.6579	784	1,185
6	288	34	443	52	1,653	631.3	0.2680	2,356	0.2574	0.7017	268	528
7	0		86		367	122.6	0.2343	523				

$\hat{E} = 49{,}390$

(c) Variance of terms and total estimate

i	$\hat{\sigma}^2_{\bar{z}_i}$	$\hat{\sigma}^2_{R_i}$	$\hat{\sigma}^2_{R \cdot i}$	$\hat{\sigma}^2_{M_i}$	$\left(\frac{\hat{\sigma}_{f_i}}{\hat{f}_i}\right)^2$	$\hat{\sigma}^2_{N_i}$	$\hat{\sigma}^2_{S_i}$	$\hat{\sigma}^2_{u_i}$	$\hat{\sigma}^2_{B_i}$	$\hat{\sigma}^2_{D_i}$
0							0.04			541,420
1			217.9			194,651	0.002030		2,141,663	4,631,018
2	33.9	184.0	687.0	1,898.2	0.005044	2,094,086	0.000040	0.002072	551,652	3,583,072
3										
4	212.3	508.6	981.6	1,084.1	0.001719	489,063	0.001091	0.000538	549,599	652,768
5	193.7	1,000.2	229.8	4,032.0	0.000682	152,246	0.001435	0.000515	86,964	201,310
6	32.8	390.7	48.1	2,027.7	0.001991	39,280	0.002779	0.002505	20,024	89,417
7		80.9		790.7	0.010938	17,381		0.010020		

$\hat{\sigma}^2_E = 9,699,005$

[a]Upper figure is number of recoveries, lower figure is variance as estimated by $\sigma^2 = npq$.
[b]Not included in R_i.

APP. TABLE IV. Estimation of the spawning population of lower Atnarko pink salmon in 1965, Seber–Jolly method.

(a) Time periods, tagging, and recovery data

Tagging			Recovery period						
Period	Date	No.	2	3	4	5	6	7	$R_{\cdot i}$
1	Sept. 16	436	237	(59)	23	7	1	1	269
			230.2		23.0	7.0	1.0	1.0	262.2
2	Sept. 22	8138		(4917)	1141	159	23	14	1337
					1044.8	155.8	22.8	13.8	1237.2
3	Sept. 25–26	0							
4	Sept. 30	3949				1652	110	45	1807
						1306.9	105.6	43.0	1455.5
5	Oct. 3	1955					631	41	672
							486.4	39.4	525.8
6	Oct. 6	655						179	179
								148.0	148.0
7	Oct. 9	0							
$R_{i\cdot}$			237		1164	1818	765	280	
			230.2		1067.8	1469.7	615.8	245.2	

(b) Estimate of population

i	T_i	Ξ_i	$R_{i\cdot}$	$R_{\cdot i}$	C_i	$\hat{M}_i$	$\hat{f}_i$	$\hat{N}_i$	$\hat{S}_i$	$\hat{u}_i$	$\hat{B}_i$	$\hat{D}_i$
0									0.6			3,242
1	436	0		269	436			794	0.9899		14,200	14,273
2	8,138	32	237	1,337	8,229	431.6	0.0288	14,986	0.1935	0.5491	15,840	36,008
3	0				(17,185)							
4	3,949	205	1,164	1,807	13,527	1,612.0	0.0861	18,722	0.5418	0.7225	5,408	7,347
5	1,955	194	1,818	672	7,908	2,382.3	0.2299	10,362	0.4504	0.7632	2,099	3,128
6	655	101	765	179	2,754	1,134.6	0.2778	4,084	0.4053	0.6743	725	1,139
7	0		280		1,032	415.2	0.2713	1,530				

$\hat{E} = 65,142$

(c) Variance of terms and total estimate

i	$\hat{\sigma}^2_{\Xi i}$	$\hat{\sigma}^2_{R i \cdot}$	$\hat{\sigma}^2_{R \cdot i}$	$\hat{\sigma}^2_{M i}$	$\left(\frac{\hat{\sigma}_{f i}}{\hat{f}_i}\right)^2$	$\hat{\sigma}^2_{N i}$	$\hat{\sigma}^2_{S i}$	$\hat{\sigma}^2_{u i}$	$\hat{\sigma}^2_{B i}$	$\hat{\sigma}^2_{D i}$
0							0.04			1,665,073
1			262.2			29,825	0.007574		2,685,342	3,106,708
2	32.0	230.2	1,237.2	1,439.8	0.004098	2,656,110	0.000084	0.003566	679,960	4,601,548
3										
4	201.4	1,067.8	1,455.5	2,119.1	0.000788	561,873	0.000225	0.000837	296,059	587,771
5	185.4	1,469 7	525.8	3,409.0	0.000445	112,310	0.000558	0.000609	84,201	213,887
6	97.2	615.8	148.0	2,548.3	0.001052	50,571	0.003001	0.001379	77,278	214,435
7		245.2		3,669.2	0.003128	57,146				

$\hat{\sigma}^2_E = 10,389,422$

These data lead to the estimates of 49,390 fish for the upper Atnarko and 65,142 fish for the lower Atnarko spawning populations. The degree of confidence in these estimates is reflected by the probable error ($\pm 0.6745\ \sigma$) which for the upper Atnarko estimate is $\pm 4.3\%$ and for the lower Atnarko is $\pm 3.3\%$. The possible error is taken as $\pm 2\ \sigma$ which is $\pm 12.6\%$ for the upper Atnarko and $\pm 9.9\%$ for the lower Atnarko of the estimated values. These estimates may be compared with those of App. Table I for the same spawning areas derived by crude census methods. The crude estimate for the upper Atnarko is 132% of the Seber–Jolly estimate, and the crude estimate for the lower Atnarko is 111% of the Seber–Jolly estimate. For both spawning sections together, the crude estimate (138,116) is 121% of the Seber–Jolly estimate (114,532).

The Seber–Jolly method must underestimate the spawning population because it is restricted to those carcasses that become available to sampling. It appears, then, that the crude estimates (App. Table I) are reasonable estimates of the spawning populations of pink salmon in the Atnarko River and I have assigned a probable error of $\pm 10\%$ ($\hat{\sigma} = 14.8\%$) of the estimate as a sufficiently large basis for estimating the variance.

On the suggestion of Dr Paulik, Dr Pienaar was asked to compare the methods of estimating variance used herein with that proposed by Dr Paulik. His report is quoted verbatim.

"In order to evaluate the efficiency of the much simplified variance estimation procedure used by the author, asymptotic variances of the estimates as derived by Jolly (1965) have been computed for data presented in App. Table III.
Thus 1)

$$\text{Var}\ (\hat{M}_i) = (M_i - R_{i\cdot})\ (M_i - R_{i\cdot} + T_i)\left(\frac{1}{R_{\cdot i}} - \frac{1}{T_i}\right) + M_i - \sum_{j=0}^{i-1} \frac{M_{i(j)}^2}{B_j}$$

where $M_{i(j)}$ = excepted number of marked carcassess in the population at time i which first joined the population between times j and $j + 1$, i.e. which are members of B_j $(1 \leq j \leq i - 1)$. Substituting observed and estimated values, the following two estimates of Var $(\hat{M}_i)$ were computed for the purpose of comparison:

$$\text{Var}\ (\hat{M}_2) = 1889$$

$$\text{Var}\ (\hat{M}_5) = 4132.$$

$$2)\ \text{Var}\ (\hat{N}_i) = N_i(N_i - C_i)\left[\frac{M_i - R_{i\cdot} + T_i}{M_i}\left(\frac{1}{R_{\cdot i}} - \frac{1}{T_i}\right) + \frac{1-f_i}{R_{i\cdot}}\right] + N_i - \sum_{j=0}^{i-1} \frac{N_{i(j)}^2}{B_j}$$

where $N_{i(j)}$ = expected number in the population at time i which first joined the population between times j and $j + 1$.

$$\text{Thus Var}\ (\hat{N}_2) \approx 1{,}470{,}000$$

$$\text{Var}\ (\hat{N}_5) \approx 61{,}500$$

$$3)\ \text{Var}\ (\hat{S}_i) = S_i^2\left[\frac{(M_{i+1} - R_{i+1\cdot})\ (M_{i+1} - R_{i+1\cdot} + T_{i+1})}{M_{i+1}^2}\left(\frac{1}{R_{\cdot i+1}} - \frac{1}{T_{i+1}}\right) + \right.$$

$$\left. + \frac{M_i - R_{i\cdot}}{M_i - R_{i\cdot} + T_i}\left(\frac{1}{R_{\cdot i}} - \frac{1}{T_i}\right) + \frac{1-S_i}{M_{i+1}}\right]$$

so that

$$\text{Var } (\hat{S}_2) \approx 0.000035$$

$$\text{Var } (\hat{S}_5) \approx 0.001120$$

$$4)\ \text{Var } (\hat{B}_i) = \frac{B_i^2(M_{i+1} - R_{i+1\cdot})(M_{i+1} - R_{i+1\cdot} + T_{i+1})}{M_{i+1}^2}\left(\frac{1}{R_{\cdot i+1}} - \frac{1}{T_{i+1}}\right) +$$

$$+ \frac{M_i - R_{i\cdot}}{M_i - R_{i\cdot} + T_i}\left(\frac{S_i T_i (1-f_i)}{f_i}\right)^2\left(\frac{1}{R_{\cdot i}} - \frac{1}{T_i}\right) +$$

$$+ \frac{(N_i - C_i)(N_{i+1} - B_i)(1-f_i)(1-S_i)}{M_i - R_{i\cdot} + T_i} + N_i N_{i+1} - C_{i+1})\left(\frac{1-f_{i+1}}{R_{i+1\cdot}}\right) +$$

$$+ S_i^2 N_i (N_i - C_i)\left(\frac{1-f_i}{R_{i\cdot}}\right)$$

so that

$$\text{Var } (\hat{B}_2) \approx 263{,}700$$

$$\text{Var } (\hat{B}_5) \approx 10{,}600.$$

"It is clear that the author's simplified procedure led to considerable overestimates of the variances of $\hat{N}_i$ and $\hat{B}_i$, resulting in a standard error of about 1.5 times as large, on the average.

"The asymptotic variance of $\hat{E}$ is given by

$$\text{Var } (\hat{E}) \sum_{i=0}^{I-1} \text{Var } (\hat{D}_i) + 2 \sum_{i=1}^{I-1} \text{Cov } (\hat{D}_{i-1}, \hat{D}_i)$$

since

$$\text{Cov } (\hat{D}_j, \hat{D}_i) = 0 \text{ for } j < i-1$$

$$\text{Var } (\hat{D}_i) \approx S_i^{-1} \text{ Var } (\hat{B}_i) + \left(\frac{B_i^2 S_i^{-3}}{4}\right) \text{Var } (\hat{S}_i) - B_i S_i^{-2} \text{ Cov } (\hat{B}_i, \hat{S}_i)$$

so that

$$\text{Var } (\hat{D}_2) \approx 1{,}810{,}000$$

$$\text{Var } (\hat{D}_5) \approx 26{,}400.$$

"The Cov $(\hat{D}_{i-1}, \hat{D}_i)$ appeared to be either negative or small so that Var $(\hat{E})$ was also overestimated, resulting in a standard error of approximately 1.5 that given by the procedure proposed by Dr. Paulik."

Thus it is clear that larger variances have been estimated by using Deming's method and ignoring covariances. The larger variances are, however, sufficiently small to allow the mortality comparisons, for which the spawning populations were a necessary component. Under the field conditions of this study, the possibility of mistakes and violation of assumptions, and without the benefit of experimental repetition or controls, a preference for the larger estimates of variance in this particular application would appear justified.

NOTES

Pathogenicity of *Gaffkya homari* for the Crab *Cancer irroratus*

Gaffkya homari is the causative agent of gaffkemia, the fatal septicemic disease of the lobsters *Homarus americanus* and *H. vulgaris* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; Roskam, 1957). The literature reviewed by Stewart et al. (1966) illustrates this microorganism's ubiquitousness; however, only the preliminary report of Bell and Hoskins (1966) examines the question of its pathogenicity for other marine species. Their results suggest that *G. homari* might be pathogenic for *Cancer magister* and to a lesser extent for *Pandalus platyceros*. *Gaffkya*-like microorganisms have been isolated from the Atlantic crab, *Cancer irroratus* (J. Graham, personal communication), but no experiments were done to show their pathogenicity for this or other local crab species. Our study, applied to *C. irroratus* which occupies the same grounds as the lobster (Wilder, 1966), showed that *G. homari* can be pathogenic for this species.

The pathogenicity of *G. homari* for the lobster is related to its extremely good growth in the lobster's hemolymph (Rabin, 1965; Cornick and Stewart, 1968), i.e. the hemolymph does not exhibit any bactericidal or bacteriostatic properties effective against this microorganism. Accordingly, crab hemolymph was examined to discover whether it had any inhibitory effects against *G. homari*. Preparation of the sterile serum and the methods used were identical to those described previously (Cornick and Stewart, 1968). The growth of both strains of *G. homari* (ATCC 10400 and Rabin's) was essentially the same in the serum from each of three crab species (*C. irroratus*, *Cancer borealis*, and *Hyas coarctatus*). A typical growth curve is illustrated in Fig. 1. The excellent growth compares favourably with the growth of this microorganism in lobster serum (Cornick and Stewart, 1968) and suggests that these three crab species would be susceptible to *G. homari*.

Experiments were set up to examine the pathogenicity of *G. homari* for *C. irroratus*. The *G. homari* suspension used for infection was prepared from infected lobsters as outlined previously (Cornick and Stewart, 1968). Twelve crabs (experimental) (mean weight, 169 g), acclimated at 15 C for 2 weeks, received a dose of 6.4×10^6 bacteria/kg body weight, injected into the hemolymph. These were held unconfined and unfed in recirculated sea water at 15 C. A corresponding number of crabs (controls) received injections of sterile 3% NaCl.

Cannibalism resulted in an excessive number of deaths in both the control and experimental groups of animals. After 49 days, however, four control

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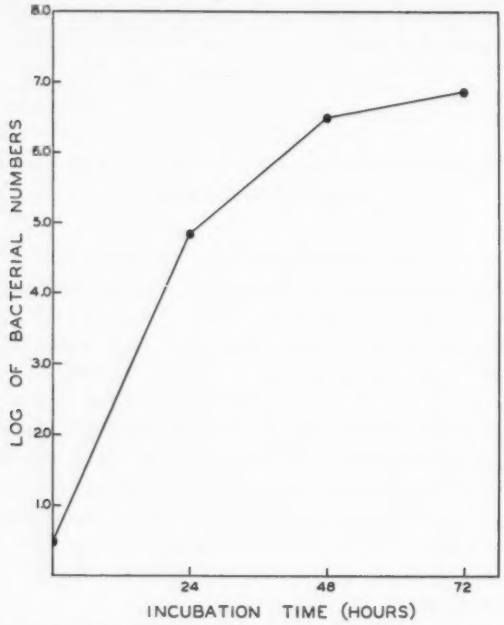

FIG. 1. Typical in vitro growth at 15 C of *G. homari* in sera prepared from *C. irroratus*, *C. borealis*, or *H. coarctatus*

and three experimental crabs were still alive. The three experimental crabs were heavily infected, containing approximately 10^9 bacteria/ml of hemolymph. These animals were then used as a source of *G. homari* for determining whether the virulence of the organism had been altered by its prolonged incubation in the crab.

The *G. homari* suspension was prepared from the infected crabs in exactly the same manner as the original suspension was prepared from the infected lobsters. This suspension was injected into 10 lobsters (*H. americanus* Milne Edwards, weighing approximately 500 g each) (experimental) with 10 others injected with sterile 3% NaCl serving as controls, all maintained at 15 C. The details of the procedure and conditions for this test have been described previously (Cornick and Stewart, 1968). No deaths or infections occurred among the controls; the mean time to death (MTD) for the experimental lobsters was 18 days, i.e. unchanged from previous trials in which the passage was from lobster to lobster (Cornick and Stewart, 1968). Passage through the crab had not altered the organism's virulence for the lobster.

The trials with crabs were repeated (Table I) using conditions designed to prevent cannibalism. All crabs (mean weight 83 g) were held in separate clear plastic boxes with holes drilled in the surfaces to allow free flow of water.

TABLE I. *Cancer irroratus* mortalities.[a]

Group	No. of crabs	Treatment	No. dead	No. surviving	No. dead infected	No. surviving infected
A	15	Injected with *G. homari*	12	3	9	3
B	15	" " sterile 3% NaCl	8	7	1	0
C	30	" " sterile 3% NaCl	13	17	0	0

[a]Mortalities refer to the number of deaths recorded by the termination of the experiment on the 80th day. Temperature, 15 C. Groups A and B shared the same tank, group C was held in a separate tank. All crabs were housed in individual cages in these tanks. Criterion for infection was isolation and identification of the pathogen from the hemolymph at death or at the termination of the experiment.

In addition, the crabs were fed twice a week on an alternating diet of herring and beef liver. Fifteen crabs (group A) received a dose of 3×10^6 cells of *G. homari*/kg body weight, injected into the hemolymph. These were maintained in the same tanks with another group of 15 crabs (group B) which received 3% NaCl solution injections, so that although they were not in direct contact they were all in close proximity and shared the same water. Another group of 30 crabs (group C) which received 3% NaCl injections served as controls and were maintained in a separate tank. Apparently conditions were still less than ideal since an excessive number of deaths occurred in the control groups (Table I). This experiment was terminated after 80 days. A chi-square analysis, using the two-way classification method of Dixon and Massey (1951), comparing the number surviving and dead for both group A and groups C + B crabs showed that significantly more deaths ($X^2 = 4.26$, $P = 0.05$) occurred in the infected than in the control groups and that the number of those dying with the infection in group A was significantly higher than those in group B. Thus *G. homari* is moderately pathogenic for *C. irroratus*.

The much milder pathogenic effect of *G. homari* upon *C. irroratus* was shown by the increased MTD for this species (42 days) in contrast to that for the lobster (18 days). Additional evidence of the mildness of the infection was provided by the re-isolation of *G. homari* from only 12 (9 dead, 3 alive) of the 15 crabs (group A) originally injected with it and the pathogen was not readily transmitted to intact crabs since it was isolated from only 1 of the 15 control crabs (group B) sharing the same water with the infected animals.

The agglutinin(s) possessed by this crab may account in part for the much milder infection. Cornick and Stewart (1968) reported that an agglutinin(s) in lobster hemolymph serum was inactive against *G. homari*. When the same methods were applied to the crab we found that four out of five individual *C. irroratus* hemolymph sera agglutinated this organism although the agglutinin apparently was not strong enough to prevent growth in vitro. It is possible that continued or enhanced production of the agglutinin in the

infected animal could contribute to the prolonged MTD or play a considerable role in the elimination of the pathogen.

The prolonged MTD for *C. irroratus* (42 days at 15 C) indicates the potential of the crab as a reservoir of infection for the lobster. The importance of the sea water temperature is to be appreciated in this connection. Our unpublished data for the lobster show that the MTD is approximately doubled if we decrease the water temperature from 15 to 10 C; temperatures lower than this effect a much greater increase in the MTD. Although we do not have comparable data for the crab, if it were assumed that the same situation exists, the normal seasonal temperatures could result in a several-fold multiplication of the MTD of 42 days. Records of seawater temperatures for the Halifax, N.S. area indicate that temperatures of 10–15 C occur only during the months of August, September, and October; July, November, and December temperatures are 8 C or less; June temperatures are less than 6 C with temperatures for the other 5 months fluctuating between 1 and 4 C. Thus, because not all animals would be infected at the same time in nature, the period of elevated temperatures would not be sufficiently long for all of the crabs to die and consequently survivors could remain as a source of infection for the next warm-water season.

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Early Larvae of the Canary Rockfish, *Sebastodes pinniger*

Rockfishes of the genus *Sebastodes* are ovoviviparous or oviparous (if oviparous, the young are hatched immediately after the ova are extruded from the female). Pigment patterns on advanced embryos or early larvae from a gravid female rockfish of known identity aid in identifying more advanced larvae. Using this approach, Morris (1956) and Delacy et al. (1964) described early larvae of 13 species of *Sebastodes* from the eastern Pacific Ocean. This note presents the first description of early larvae of the canary rockfish, *Sebastodes pinniger* (Jordan and Gilbert), 1880.

A 57-cm gravid female canary rockfish was caught in a Cobb pelagic trawl on January 12, 1965, off the Washington coast at 47°47′N lat, 124°52′W long. Eggs containing advanced embryos were oozing from the fish; some of them were collected and placed in a container of sea water. At the laboratory 2 days later, 75 eggs were transferred to petri dishes containing sea water (salinity 32‰, temperature 10 C); the rest were preserved in formalin. Most of the live embryos were still in the eggs, but a few had hatched, possibly as a result of handling. The ellipsoidal preserved ova had average measurements of 1.45, 1.23, and 0.84 mm. In dorsal aspect 145 embryos curved to the left, and 130 curved to the right within the eggs.

All of the live embryos had hatched by January 15; the larvae remained on the bottom of the petri dishes and moved only occasionally. On January 21 the jaws moved but not the eyes. By January 22 the larvae had become fairly active and could maintain position in mid water for some time. In the shallow dishes in which they were kept, however, they soon sank to the bottom. No attempt was made to feed the larvae and except for a few that died from natural causes, all were killed and preserved by February 1.

The yolk sacs had decreased in size but were still present in the larvae preserved on February 1. The standard length had changed little. The average for 23 larvae preserved on January 14 was 3.6 mm (3.1–3.9 mm); the average for 6 larvae preserved on January 29 was 3.8 mm. Pectoral fin length increased from about 0.1 mm on January 14 to about 0.5 mm on January 29.

The amount of pigment on the larvae gradually increased during the 2-week period after hatching. Until January 18, the only melanophores were an irregular double row along the ventral body surface from the 18th to 22nd myomeres and a small patch above the yolk sac near the anus (Fig. 1). Larvae preserved on January 18 and 19 showed one or two melanophores along each side above the ventral row, apparently migrating dorsally. These lateral melanophores were lacking on larvae preserved at later dates. By January 22 a short, irregular double row of melanophores was present along the dorsal body surface on the 19th–21st myomeres, and single melanophores were

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visible at the tip of the lower jaw and in the hypural region. After January 26 one to four melanophores were present on the ventral part of the yolk sac; by February 1 the pectoral fins carried several small melanophores.

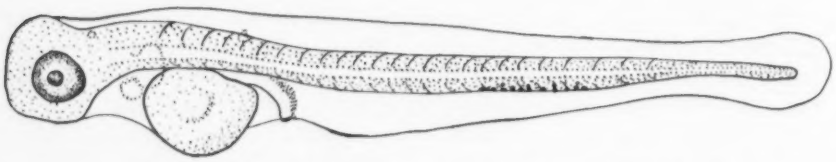

FIG. 1. Pigment pattern of newly hatched canary rockfish.

Length of the ventral pigment row varied on larvae preserved from January 28 to February 1; the most anterior melanophores were on myomere 14–18 and the most posterior on myomere 21 or 22.

Immediately after hatching, the larvae resembled those of *S. paucispinis* as described by Morris (1956). After 2 weeks of development, and an increase in the pigment, larvae of *S. pinniger* (Fig. 2) resembled newly hatched *S. jordani* of Morris (1956), although *S. pinniger* had shorter dorsal and ventral rows of melanophores.

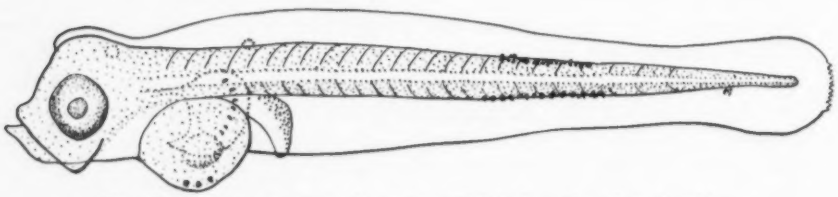

FIG. 2. Pigment pattern of canary rockfish 2 weeks after hatching.

A number of small serrations had developed along the posterior edge of the caudal finfold of specimens preserved on and after January 26; these serrations could be seen on anesthetized, living specimens and thus cannot be considered an artifact of preservation.

Morris (1956) mentioned a "small discoidal indentation" on the dorsal finfold of his specimens. Two pairs of such structures were observed on *S. pinniger* — one pair on the dorso-lateral body surface at about the fifth myomere and the other just behind and slightly above the eyes.

The features considered by Morris (1956) and Delacy et al. (1964) in their descriptions of other *Sebastodes* larvae are presented below for *S. pinniger*. The measurements given are averages of 10 specimens of newly hatched larvae; the pigment patterns are for newly hatched and 2-week-old larvae.

Measurements of newly hatched larvae: standard length, 3.61 mm; diameter of eye, 0.27 mm; tip of snout to anus, 1.24 mm; tip of snout to posterior edge of otic capsule, 0.51 mm; body depth in region of heart, 0.53 mm.

Larval Pigment Patterns

	Newly hatched larvae	2-week-old larvae
Ventral pigment row present	+	+
Ventral pigment row stops short of anus	+	+
Ventral pigment row multiple or irregular	+	+
Ventral row stops short of anus by as much as four myomeres	+	+
Ventral row usually with fewer than 16 melanophores	+	+
Dorsal pigment row present	−	+
Dorsal row stops short of anus	−	+
Dorsal row multiple or irregular	−	+
Head or nape with at least some pigment	−	−
Head with two to five melanophores	−	−
Head with more than five melanophores	−	−
Lower jaw with some pigment	−	+
Hypural region with pigment spot(s)	−	+
Pectoral fins with some pigment	−	+

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Reinterpretation of Isopropyl Alcohol Retention in Fish Protein Concentrate[1]

A recent communication described the determination of isopropyl alcohol in fish protein concentrate (Ackman et al., 1967). The rigid retention of isopropyl alcohol under extreme drying conditions observed in this work was ascribed to encapsulation by a surface "shell" of protein denatured or hardened through drying (Ackman et al., 1967). Such a "shell" should have a discrete thickness and an attempt has now been made to demonstrate the "shell" microscopically. Samples of fish protein concentrate were directly embedded in paraffin wax without further processing, and were sectioned at 4 μ. The sections were stained with haematoxylin and eosin and were photographed. The particles varied widely in size and shape (Fig. 1). Many frayed pieces of muscle fibre were visible but there was no evidence for any "shell" of protein.

Menting and Hoogstad have recently investigated the transfer of volatile organic materials in the drying of droplets of an aqueous solution of maltodextrin (1967a) and in sorption by coatings of aqueous solutions of maltodextrin on Chromosorb W (1967b). The conclusions of these authors are that there is operative a selective membrane, lattice, or sieve effect which permits passage of water molecules and volatile organic molecules above a limiting proportion of water to solids. Below this level the organic molecules will not pass through the membrane or lattice formed of solids and water.

This process should be operative with membranes not detectable by visible light microscopy. We suggest that in the apparent absence of a "shell" of dried protein the partially hydrated membrane effect accounts for the trapping of isopropyl alcohol within particles of fish protein concentrate. As demonstrated by Ackman et al. (1967) a dried fish protein concentrate sample with a natural degree of hydration when subjected to vigorous dehydration lost part of its isopropyl alcohol as well as water, but rapidly re-absorbed atmospheric water (4.6%) in almost exactly the same proportion as that of water lost (4.8%) on drying. Thus the proportion of water to protein apparently has a natural level which is low enough to normally block passage of isopropyl alcohol. This level may be similar for low-polarity organic particles in general, since Gur-Arieh et al. (1967) have demonstrated a transition point in the absorption of water on flour particles at 7% water. These authors believe that this point represents the completion of a monomolecular layer of water.

"Neutral" volatile components other than isopropyl alcohol (Wick et al., 1967) may also be trapped in fish protein concentrate particles by the membrane effect, although this may not be the only mechanism for polar materials such as amines.

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(FIG. 1 *opposite*)

FIG. 1. Particles of fish protein concentrate (prepared from cod muscle) sectioned and stained as described in text.

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Food of the Buffalo Sculpin, *Enophrys bison*

According to Clemens and Wilby (1961) *Enophrys bison* (Girard) is common from southern California to the Gulf of Alaska and feeds upon shrimps, crabs, amphipods, mussels, small fishes such as herring, young salmon, seaperches, and sandlances as well as considerable amounts of sea lettuce, *Ulva*.

The objective of this study was to examine the food of the buffalo sculpin over an extended period of time.

This study was conducted in the eelgrass, *Zostera marina*, association near Southport Channel in Humboldt Bay, Humboldt County, Calif. Habitats sampled included eelgrass beds on mud flats exposed at low tides and eelgrass beds adjacent to mud flats but never exposed at low tide. Analysis of trawl data over the past 8 years revealed that sculpins were only taken in quantity when hauls were made in the eelgrass, indicating that *E. bison* probably is closely associated with this habitat. Besides buffalo sculpins, other fishes captured, in order of decreasing abundance, were: lemon sole (*Parophrys vetulus*), shiner seaperch (*Cymatogaster aggregata*), speckled sanddab (*Citharichthys stigmaeus*), and white seaperch (*Phanerodon furcatus*).

Methods.—Buffalo sculpins were collected with a small experimental otter trawl operated aboard the R.V. *Seagull* during February 1963 and August and October 1964. The trawl is a Gulf of Mexico shrimp trawl similar to that described by Schaefers and Johnson (1957) and by Alverson et al. (1960). Fishing time on the bottom was approximately 10 min on all hauls. Upon capture the sculpins were preserved immediately in 10% formalin. In the laboratory the stomach contents were removed and examined with a binocular dissecting scope. The organisms found were identified to the lowest taxon possible, with keys by Light et al. (1957), Barnard (1954), and Hartman and Reish (1950), depending upon their state of digestion. The volume of the organisms was obtained for each taxon by measuring the water displacement. The standard length of each fish was recorded to the nearest millimeter.

The fish were divided into three size groups, 0–45, 46–65, and 66–180 mm, for stomach content analysis. Several larger specimens captured along the open rocky coast were considered separately. For each size-group the frequency occurrence, per cent volume, and average number of organisms were considered. Frequency occurrence is expressed as the percentage of all fish in the sample having a particular food item. Per cent volume is the percentage each organism contributed to the total volume of organisms eaten. The average number of organisms per fish is the quotient of the total number of organisms in the taxon divided by the number of fish having this organism in the gut.

Results.—In the 0–45-mm size-group, gammarid amphipods (*Ampithoe* spp.) had the highest frequency occurrence in all months and was third in per cent volume in October (Table I). Caprellid amphipods were second highest

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TABLE I. Digestive tract contents of buffalo sculpin in size-group 0–45 mm. FO = frequency occurrence; Tr = less than 1.0.

Item	Feb. 1963			Aug. 1964			Oct. 1964			Total		
	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.
Ulva lobata	5	2	–	–	–	–	25	22	–	8	6	–
Amphipoda												
Gammaridea[a]	20	4	1.7	100	9	2.4	33	4	2.5	30	5	2.1
Ampithoe spp.	28	10	1.7	20	5	1.0	42	19	2.2	30	11	1.8
Nototropis spp.	–	–	–	–	–	–	17	Tr	1.5	3	Tr	1.5
Caprellidea												
Caprella spp.	9	Tr	1.3	40	48	1.5	42	22	2.0	18	12	1.6
Isopoda												
Idothea spp.	–	–	–	20	Tr	1.0	–	–	–	1	Tr	1.0
Paranthura sp.	–	–	–	–	–	–	8	Tr	1.0	2	Tr	1.0
Munna spp.	–	–	–	40	Tr	1.5	8	4	4.0	5	Tr	2.3
Pelycepoda												
Protothaca sp.	2	Tr	1.0	–	–	–	8	Tr	1.0	3	Tr	1.0
Macoma siphons	–	–	–	–	–	–	25	Tr	1.0	5	Tr	1.0
Chelifera												
Tanais spp.	5	Tr	1.0	20	Tr	1.0	–	–	–	5	Tr	1.0
Copepoda[a]	–	–	–	–	–	–	8	Tr	1.0	2	Tr	1.0
Mysidacea[a]	2	Tr	1.0	–	–	–	8	Tr	1.0	3	Tr	1.0
Hydrozoa												
Obelia spp.	20	10	–	–	–	–	8	7	–	17	8	–
Gastropoda												
Nassarius fossatus	23	4	2.5	20	Tr	3.0	8	4	2.0	20	3	1.5
Polychaeta[a]	5	4	1.0	–	–	–	–	–	–	3	2	1.0
Eulalia aviculiseta	12	4	1.4	20	24	1.0	–	–	–	10	7	1.3
Eunereis sp.	2	7	1.0	–	–	–	–	–	–	2	5	1.0
Detritus	63	54	–	20	5	–	8	7	–	48	36	–
Unknown	2	1	–	20	9	–	33	11	–	10	5	–
No. of fish		43			5			12			60	
No. empty		2(5%)			0			0			2(3%)	

[a]Genus not known.

in frequency occurrence and highest in per cent volume in August and October. Immature specimens of the gastropod *Nassarius fossatus* were nearly as high as the amphipod genus *Ampithoe* in frequency occurrence in February. Sea lettuce, *Ulva lobata*, was not taken in any quantity until October.

In 46–65-mm size-group, amphipods (both gammarids and caprellids) were highest in frequency occurrence in February (Table II). In August and October, *Ulva lobata* and the amphipods *Caprella* spp. were highest in frequency occurrence and *Ulva lobata* was highest in per cent volume. Unidentified amphipods had the third highest frequency occurrence in both these months. Juvenile *Nassarius fossatus* again made up in a high proportion of the diet in the February sample.

TABLE II. Digestive tract contents of buffalo sculpin in size-group 46–65 mm. FO = frequency occurrence, Tr = less than 1.0.

Item	Feb. 1963			Aug. 1964			Oct. 1964			Total		
	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.
Ulva lobata	4	2	–	64	38	–	73	51	–	28	31	–
Amphipoda												
Gammaridea[a]	25	2	1.7	55	3	2.0	55	9	6.2	36	6	3.5
Ampithoe spp.	29	8	1.6	46	8	2.4	9	1	8.0	26	4	2.3
Nototropis spp.	–	–	–	9	Tr	2.0	5	Tr	1.0	2	Tr	1.5
Elasmopus sp.	–	–	–	–	–	–	5	Tr	1.0	1	Tr	1.0
Caprellidea												
Crapella spp.	29	8	1.8	73	13	2.8	64	10	4.7	43	10	3.1
Isopoda[a]	2	Tr	1.0	–	–	–	–	–	–	1	Tr	1.0
Idothea spp.	4	1	1.0	9	Tr	2.0	5	Tr	4.0	5	1	2.0
Munna spp.[a]	–	–	–	9	Tr	2.0	–	–	–	1	Tr	2.0
Flabellifera[a]	–	–	–	–	–	–	14	Tr	2.0	3	1	2.0
Pelycepoda												
Protothaca sp.	2	Tr	1.0	–	–	–	9	Tr	1.0	3	Tr	1.0
Macoma sp.	2	Tr	1.0	–	–	–	–	–	–	1	Tr	1.0
Macoma siphons	4	Tr	1.0	–	–	–	14	Tr	1.0	6	Tr	1.0
Copepoda[a]	2	Tr	1.0	9	Tr	2.0	–	–	–	2	Tr	1.5
Mysidacea[a]	–	–	–	9	Tr	1.0	–	–	–	1	Tr	1.0
Hydrozoa												
Obelia spp.	9	3	–	36	19	–	5	Tr	–	11	4	–
Gastropoda												
Nassarius fossatus	27	3	2.7	–	–	–	–	–	–	17	1	2.7
Foraminifera[a]	2	Tr	1.0	–	–	–	–	–	–	1	Tr	1.0
Pycnogonida												
Phoxichilididae[a]	2	Tr	1.0	–	–	–	5	Tr	1.0	3	Tr	1.0
Polychaeta[a]	4	1	1.0	–	–	–	5	Tr	5.0	3	1	2.3
Eulalia aviculiseta	9	1	1.2	–	–	–	5	Tr	1.0	7	Tr	1.2
Glycera sp.	2	9	1.0	–	–	–	–	–	–	1	3	1.0
Nereis sp.	–	–	–	–	–	–	5	25	1.0	1	12	1.0
Decapoda												
Brachyura[a]	2	1	1.0	–	–	–	–	–	–	1	Tr	1.0
Cancer sp.	2	Tr	1.0	–	–	–	–	–	–	1	Tr	1.0
Caridea												
Crangon sp.	5	25	1.0	–	–	–	–	–	–	3	9	1.0
Detritus	60	36	–	18	1	–	9	1	–	42	13	–
Unknown	–	–	–	18	19	–	9	3	–	5	4	–
No. of fish		55			11			22			88	
No. empty		1(2%)			0			0			1(1%)	

[a]Genus not known.

In the 66–180-mm size-group, gammarid and caprellid amphipods had the highest frequency occurrence in February (Table III) The August sample with only two fish was too small to be significant. In October, *Ulva lobata*

TABLE III. Digestive tract contents of buffalo sculpin in size-group 66–180 mm. FO = frequency occurrence, Tr = less than 1.0.

Item	Feb. 1963			Aug. 1964			Oct. 1964			Total		
	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.	% FO	% vol.	$\bar{x}$ no.
Ulva lobata	–	–	–	50	30	–	92	81	–	67	56	–
Porphyra spp.	–	–	–	–	–	–	3	Tr	–	2	Tr	–
Amphipoda												
Gammaridea[a]	31	Tr	2.0	–	–	–	5	1	7.0	12	1	3.7
Ampithoe spp.	8	Tr	2.0	–	–	–	38	2	4.0	29	1	3.9
Elasmopus spp.	–	–	–	–	–	–	22	Tr	1.4	15	Tr	1.4
Nototropis spp.	–	–	–	–	–	–	24	Tr	3.2	17	Tr	3.2
Caprellidea												
Caprella spp.	46	4	4.0	50	Tr	6.0	76	6	4.4	67	5	4.4
Isopoda												
Idothea spp.	–	–	–	50	16	1.0	25	2	2.7	19	4	2.1
Flabellifera[a]	–	–	–	–	–	–	5	Tr	1.0	4	Tr	1.0
Pelycepoda												
Protothaca sp.	–	–	–	–	–	–	3	Tr	1.0	2	Tr	1.0
Macoma siphons	–	–	–	–	–	–	3	Tr	2.0	2	Tr	2.0
Foraminifera[a]	8	Tr	1.0	–	–	–	–	–	–	2	Tr	1.0
Hydrozoa												
Obelia spp.	15	3	–	50	12	–	16	Tr	–	17	3	–
Gastropoda												
Nassarius fossatus	15	Tr	1.0	–	–	–	14	Tr	1.0	13	Tr	1.0
Polychaeta												
Eunereis sp.	8	35	1.0	–	–	–	–	–	–	2	7	1.0
Decapoda												
Caridea												
Crangon sp.	15	5	1.0	50	39	2.0	8	2	1.0	12	10	1.2
Anomura[a]	–	–	–	–	–	–	3	Tr	1.0	2	Tr	1.0
Brachyura												
Cancer productus	–	–	–	–	–	–	3	4	1.0	2	Tr	1.0
Cancer sp.	15	14	1.0	–	–	–	–	–	–	4	4	1.0
Detritus	46	39	–	–	–	–	16	Tr	–	23	8	–
Unknown	–	–	–	50	3	–	11	2	–	10	1	–
No. of fish		13			2			37			52	
No. empty		1(8%)			0			0			1(2%)	

[a]Genus not known.

had the highest frequency occurrence and per cent volume and the amphipods *Caprella* spp. were second highest in frequency occurrence. Gammarid amphipods and isopods (*Idothea* spp) were also prominent in the diet during October.

In all size-groups the polyp stage of the hydrozoan *Obelia* spp. showed up frequently in the diet.

Discussion.—Allen (1941) stated that size, appearance, habitats, and taste of potential food organisms most likely determine their abundance in the digestive tracts of fishes. Because the habits of potential food items determine their vulnerability to fishes, an attempt will be made to relate the results of the stomach analysis to the known habits of the food organisms wherever possible.

Gammarid amphipods: Unfortunately little is known of the habits of gammarids of the Pacific coast. This is most likely the result of their difficult taxonomy. *Ampithoe* spp. comprised a considerable portion of the gammarids found. Many of the ampithoids are burrowers, but some are not and can be found moving rapidly about the bottom. It is possible that the ampithoids in Humboldt Bay are non-burrowers and that this would explain their high numbers in the diet of the sculpins. The high vulnerability of gammarids in general is probably due to their high availability and abundance. Their small size makes them an excellent food source for the smaller-sized cottids.

Caprellid amphipods: Caprellids are found on algae and eelgrass on the mud flats. As with gammarid amphipods their high vulnerability is most likely due to their availability and abundance. It would appear that the caprellids are preyed upon by all size-groups of *E. bison* and during most of the year. It is also possible that caprellids are ingested along with *Obelia* as they are often found on the *Obelia* colonies.

Nassarius fossatus: This gastropod contributed to the food of the smaller fish in February and possibly supplemented their diet when other food sources such as *Ulva* were unavailable.

Ulva lobata: The life cycle of *Ulva* consists of two alternating isomorphic generations; one keeps the species going over the winter and the other flourishes over the summer months. The heavy feeding on *Ulva* during August and October is possibly due to availability.

Obelia: The feeding polyps of *Obelia* spp. can be found all year in Humboldt Bay. Although *Obelia* is available it is hard to imagine there being much food value to the sculpins in this small hydrozoan.

Detritus: The high occurrence of detritus in February possibly indicates a change in feeding habits during this month which might be attributed to spawning activities.

Several larger sculpins were captured at Trinidad, about 14 miles north of Humboldt Bay. These specimens ranged in size from 180 to 230 mm in standard length. Analysis of their stomachs revealed that they had been feeding upon the caridean shrimps *Crangon* spp. and one of the specimens had eaten a small fish but identification was impossible.

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Sexual Dimorphism in Anal Fin of Brown Trout, *Salmo trutta*, and Close Relatives

In morphological studies on Salmonidae we observed an apparently undescribed type of sexual dimorphism in brown trout, *Salmo trutta*, and two closely related species. Sexual dimorphism is well marked in adults of many salmonid species, and especially in *Salmo* (Tchernavin, 1938; Vladykov, 1963; Morton, 1965; Breder and Rosen, 1966). However, in *Salmo* most manifestations of dimorphism are temporary and occur during the spawning season (Vladykov, 1931; Stoklosowa, 1966); others require morphometric studies to separate the sexes (Berg, 1948).

In brown trout and two close relatives we found that, through most of the life history, the anal fin was of two distinct shapes: falcate or notched in females and rounded or convex in males (Fig. 1). This character is externally visible, present on specimens collected at all times of the year, and evident on small fish. We examined many salmonid species and found anal fin dimorphism only in forms of brown trout, in Sevan trout, *Salmo ischchan*, and in Ohrid trout, *S. letnica* (Table I). We studied three specimens of Lake Garda trout,

TABLE I. Species of *Salmo* in which dimorphism of the anal fin was found adequate for determining the sex of specimens. All determinations were confirmed by inspection of the gonads. FL = fork length.

Species	Males		Females	
	No. of specimens	Range in FL (*mm*)	No. of specimens	Range in FL (*mm*)
Brown trout, *S. trutta trutta* Linnaeus	8	45–312	30	51–284
S. trutta labrax Pallas	2	113–222	12	100–265
S. t. macrostigma (Dumeril)	1	184	2	195–220
Sevan trout, *S. ischchan* Kessler	6	342–386	5	380–393
Ohrid trout, *S. letnica* (Karaman)	10	56–185	6	49–175

S. carpio, and found that the anal fin was falcate; examination of the gonads confirmed that the fish were females. No male specimens were available for study.

To test the dimorphism we separated the "males" and "females" on the basis of the anal fin and then examined the gonads to check the accuracy of the prediction. All specimens of brown, Sevan, Ohrid, and Lake Garda trout that we examined were correctly sexed by means of the anal fin.

We measured the lengths of the branched anal rays to determine which rays were responsible for the dimorphism. In brown and Sevan trout, the

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depth of the notch (distance from the tip of the shortest branched ray to a line joining the tips of the longest anterior and posterior branched rays) in the female anal fin was compared with the horizontal diameter of the eye. These studies indicated that the notch in the female anal fin is caused by a greater difference in length between the intermediate (5 and 6) and the anterior and posterior branched anal rays in females than in males. In Sevan and brown trout the depth of the notch in females is $\frac{1}{6}$ (17%) of the horizontal diameter of the eye.

Although measurements of the branched anal rays and the notch verified the dimorphic nature of the anal fin, illustrations readily demonstrate that, in practice, measurements are unnecessary. The dimorphism is obvious in fishes over 150 mm fork length (Fig. 1, 2). In smaller specimens the dimorphism is not as distinct as in the larger fishes, but it is still evident (Fig. 3).

Sexual dimorphism of a similar nature has been reported in the anal fin of Hiodontidae (Kerswill, MS, 1937, p. 33; Hinks, 1943, p. 20). In this family however, the anal fin of the male is falcate and the distal margin of the anal fin in females is relatively straight; this is the opposite of the situation in the brown trout complex.

The existence of sexual dimorphism in the brown trout complex may have some systematic significance, especially in the problematical relationships of the Ohrid (Stankovič, 1960), the Sevan (Berg, 1948), and possibly the Lake Garda trout (D'Ancona and Merlo, 1958) to the brown trout. Moreover, sexual dimorphism is of great practical value in hatcheries, genetic studies, tagging operations, and other work. Valuable museum specimens can be readily sexed without mutilation.

Specimens of brown trout used in this study are from various locations in Europe and Canada; the Sevan trout are from Lake Sevan, USSR. Our specimens of Ohrid trout were raised in a fish hatchery in Minnesota, from eggs received from Lake Ohrid on the Yugoslavian–Albanian border.

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(Fig. 1–3 and References *on following pages*)

FIG. 1. Sexual dimorphism in anal fins of brown trout, *Salmo trutta*; *left*: male, 210 mm FL, anal fin length 26.5 mm; *right*, female, 213 mm FL, anal fin length 26 mm.

FIG. 2. Specimens of Sevan trout, *Salmo ischchan*, showing anal fin dimorphism; *above*, male, 342 mm FL; *below*, female, 380 mm FL.

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FIG. 3. Small specimens of Ohrid trout, *Salmo letnica*: the dimorphism is not as distinct as in large specimens but is still present; *above*, male, 57 mm FL; *below*, female, 60 mm FL.

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Very Rapid Accumulation of Hypoxanthine in the Muscle of Redfish Stored in Ice

The IMP[1]→Ino→Hx sequence of postmortem nucleotide degradation follows a common, well-defined pattern in a large variety of fish species. Hx progressively increases from values near zero at death to ultimate levels of 3–5 μmole/g or higher in the spoiled material, well beyond the limit of edibility. Hx levels, therefore, have been suggested as a monitor both of autolytic and bacterial activity (and hence are implicated either directly or indirectly in flavor changes); Hx content thus may be a useful index of storage time in ice, and possibly of quality, for an increasing number of commercially important species (Jones et al., 1964; Dyer et al., 1966; Fraser et al., 1968). Indeed, a simplified procedure for the enzymic assay of Hx, adapting it more readily for use as a quality index under field conditions, has recently been proposed (Burt et al., 1967).

In preliminary experiments, we observed an unexpectedly rapid nucleotide degradation in redfish (*Sebastes marinus*), leading to maximum accumulation of Hx after just 4–5 days storage in ice. In several lots of commercially caught redfish 2–3 days in ice on the vessel, initial samplings at landing revealed unusually high Hx contents, 1.6–2.4 μmole/g, and correspondingly low IMP levels, 0.6–2.5 μmole/g, for such fresh fish (little Ino was present). Furthermore, the rapid nucleotide catabolism continued, since IMP was completely dephosphorylated and Hx had accumulated to maximum levels of 4–5 μmole/g in just 2 days of further storage in ice.

To substantiate these findings, two more lots of redfish were obtained and sampled more frequently (Fig. 1). As in the preliminary experiments, maximum accumulation of Hx, with concomitant dephosphorylation of IMP, was reached in 4–5 days after catching. Ino did not accumulate, and the small amount initially present disappeared rapidly. The rapidity of these changes is in marked contrast to the much slower rates observed in other species so far examined (for a tabular summary of these species, see Burt et al., 1967). For example, times to maximum production of Hx have ranged from 8–10 days for Pacific Coast soles and rockfish (Spinelli et al., 1964) to more than 20 days for swordfish (Dyer et al., 1966) and halibut (Spinelli, 1967). The presence of only traces of Ino in redfish during iced storage was confirmed by the thin-layer chromatographic technique (Fraser et al., 1967). The lack of Ino accumulation has also been noted in lemon sole (Kassemsarn et al., 1963) and in petrale sole (Spinelli, 1967). This indicates that in these species inosine ribohydrolase is relatively more active than IMP phosphohydrolase.

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[1]The following abbreviations are used: IMP — 5′-inosine monophosphate; Ino — inosine; Hx — hypoxanthine.

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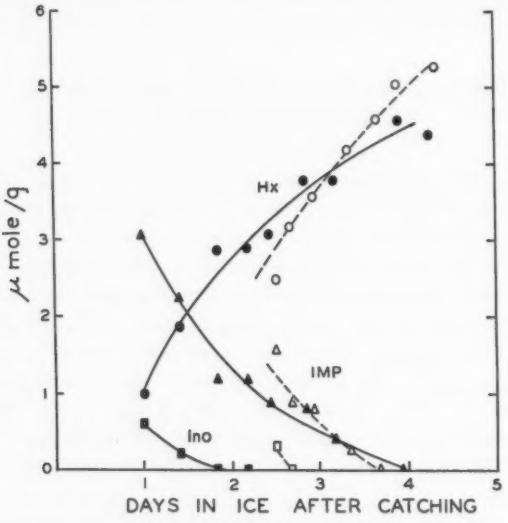

FIG. 1. IMP (▲,△) Ino (■,□), and Hx (●,○) in two different lots of redfish fillets stored in polyethylene bags buried in ice. One lot (solid symbols) had been held on ice as whole fish for 1 day on the vessel before landing and filleting (Lunenburg), and the other (open symbols) for 2–3 days (Halifax). A composite sample from six fillets, each from a different fish, was analyzed at each sampling time. IMP, Ino, and Hx were determined as described previously (Dyer et al., 1966).

The most striking finding with redfish was that Hx production reached its maximum considerably before any deterioration in eating quality became evident and long before the point of inedibility was reached. Taste-testing of several lots of redfish at 4–5 days (Hx maximum) consistently yielded taste scores of 70–80%, unchanged from the high initial levels. (Any possible Hx production in the initial samples during cooking, although unlikely (Jones, 1965), was obviously insufficient to affect flavor.) No trace of bitterness was noted; indeed, no alteration in flavor from the characteristic blandness of freshly caught redfish was evident which could be attributed to the presence of Hx at levels in excess of 4 μmole/g in the uncooked flesh. In sharp contrast, in other species where Hx measurements were accompanied by taste-panel evaluations of eating quality, maximum Hx levels were attained only *after* the limit of organoleptic acceptability was reached. For example, taste-panel rejection of mackerel occurred at an average Hx content of 2.4 μmole/g, about half the ultimate level (Fraser et al., 1968). Similar Hx levels of 2–2.5 μmole/g at the limit of acceptibility are indicated for cod (Burt et al., 1967) and herring (Hughes and Jones, 1966). With redfish, the results provide conclusive evidence that the accumulation of Hx per se was not responsible for flavor changes in the iced fish, and that factors other than the IMP-to-Hx breakdown sequence

obviously determined the limit of edibility. This was reached after about 13 days in ice, when the off-odors and flavors due to normal bacterial spoilage led to rejection by the taste panel.

We wondered whether the comparatively rapid nucleotide breakdown in redfish muscle may have been due to diffusion of enzymes from the gut contents during the 1–3 days in ice on the vessel (in commercial practice redfish are landed ungutted). It was found, however, that gutting at sea immediately after catching did not affect the subsequent rate of IMP dephosphorylation or Hx accumulation in the iced fillets (Fig. 2). This experiment also confirmed and extended our previous findings to show that maximum accumulation of

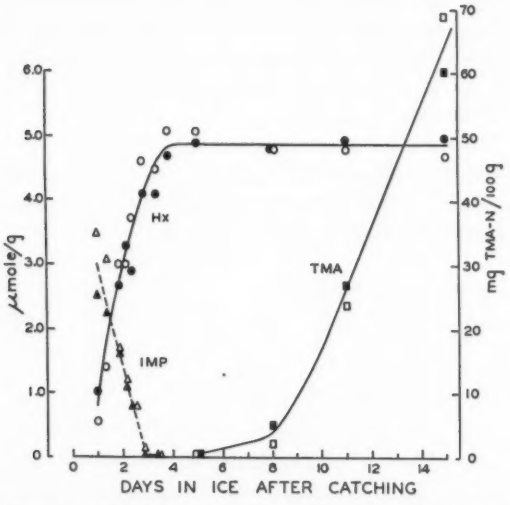

FIG. 2. IMP dephosphorylation, Hx accumulation, and development of trimethylamine (measure of bacterial spoilage) during storage in ice of redfish fillets prepared from round fish held ungutted (solid symbols) and gutted (open symbols) for 1 day in ice on the vessel. A composite sample of four to six fillets, each from a different fish, was analyzed at the sampling times indicated. IMP and Hx were determined as described previously (Dyer et al., 1966), and TMA according to Dyer (1959).

Hx, reached in 4 days and maintained at least to 15 days, preceded by several days the onset of bacterial spoilage (and hence inedibility) as measured by TMA values. We conclude that the more rapid completion of the IMP-to-Hx sequence in redfish is due simply to a more active indigenous enzyme system which is obviously not augmented by bacterial enzymes in this species. In this connection, inosine ribohydrolase, and possibly IMP phosphohydrolase, have recently been found to be much more active in redfish than in cod and halibut (J. R. Dingle, personal communication). The relatively high muscle

*p*H (about 7) measured in the redfish of the present experiment may be an important factor in the rapid rate of nucleotide breakdown, since the optimum *p*H of the enzymes involved, although not clearly defined, probably lies on the alkaline side of neutrality; low muscle *p*H values, 5.7–6.2, were suggested previously as contributing to the comparatively slow rate in mackerel and swordfish (Fraser et al., 1968), and in meat (Meyer al., et 1967).

In summary, the autolytic enzymic dephosphorylation of IMP and the production of Hx were rapidly completed in redfish (without accumulation of Ino) in just 4 days, several days before the point of inedibility was reached at the onset of bacterial spoilage. These results obviously preclude the use of Hx content as an index either of freshness or of quality in redfish. In direct contrast, maximum Hx production in other species is reached only after the point of inedibility. The redfish results also emphasize the wide variation in rate of nucleotide catabolism existing among species, and the importance of establishing the rate in an untried species before applying the Hx test to it as an index of freshness or quality.

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Method for Counting Coho Fry in Small Streams

Counting small fish in the small streams of the heavily wooded areas of British Columbia is difficult using ordinary means.

Coho fry (*Oncorhynchus kisutch*) 3.5–5 cm long are driven away rather than attracted by electrofishing. Hand-seines are constantly snagged on dead branches and twigs embedded in the stream gravel. Even if a successful sweep with a one-man hand seine is made, the rest of the fry are scattered and hard to catch.

When quick censusing of miles of stream running through dense underbrush was needed, the method of "chumming" the fry into groups was found to aid the work greatly. In the spring and summer, the coho fry and underyearlings mill about the pools in large numbers. A small ball of paper tissue thrown onto the surface will bring a quick initial response of the fry. An individual seizes the object and is pursued by numbers of fry about the pool until the object is let go or the others seize it from him, soon to reject it. Fry several feet away are attracted by the fray, the whole procedure resembling a group of dogs playing "chase" with a towel. A small piece of sandwich thrown onto the surface brought about the same response with the fry holding together much longer and attracting more and more individuals with repeated feeding. Canned salmon was used in regular work, inasmuch as quarter-pound cans were readily available from station stores. The fry ate it eagerly and the food particles were readily picked out in any size required.

The food brings the fry together and holds them because of their competitiveness. Larger coho pre-smolts and trout will often come out of hiding under banks and logs to make quick sorties into the feeding area if food is given repeatedly without disturbing the fry.

A quick count of the fry is made as they feed. If the numbers are large, extra food is tossed to one side of the pool and feeding started at the other side, the fish being counted in groups.

Exact counts are made by one man standing quietly in the pool with the hand seine while another "chums" the fry in front of the net. The netted fry are counted into a bucket and the procedure repeated. This system is an improvement on an older method of scuffling the bottom to get the fry feeding around one's feet on the disturbed insect larvae.

The method obviously is not suited for taking samples for studies of weight and stomach content. However, the amount of food introduced into a stream system is small and does not in any significant way modify the stream ecology.

A typical July count at Chef Creek on Vancouver Island was 171 fry, length 4.0–5.0 cm, average weight 1.18 g in a pool 6 × 5 m, average depth 0.28 m with the stream discharge 0.77 m^3 per second.

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Feeding Behavior of Three Oceanic Fishes (*Oncorhynchus kisutch*, *Trachurus symmetricus*, and *Anoplopoma fimbria*) from the Northeastern Pacific

During an August–September survey for saury (*Cololabis saira*) off the coasts of Washington and Oregon, biologists aboard the U.S. Bureau of Commercial Fisheries research vessel *John N. Cobb* observed coho salmon (*Oncorhynchus kisutch*), jack mackerel (*Trachurus symmetricus*), and sablefish (*Anoplopoma fimbria*) foraging on saury and blue lanternfish (*Tarletonbeania crenularis*) that had concentrated beneath floodlights at night.

The 16-kw night-lighting system consisted of six 18-ft standards spaced approximately 21 ft apart along the port side and starboard aft quarter of the ship. Two 500-w and one 1000-w photoflood fixtures were attached to each of the three forward standards on the port side and the starboard standard immediately abaft the superstructure. The aft standards on the port and starboard sides had two each 500-w and 1000-w fixtures. Two 10.5-kw, 120-v single phase auxiliary field generators furnished the power source.

Aside from the three subject species, the blue shark (*Prionace glauca*) and the soupfin shark (*Galeorhinus zyopterus*) were seen preying on saury and blue lanternfish. Only on three occasions did predators escape positive identification once they were sighted during this study.

The sharks preyed on forage fishes in the penumbra of the lighted area, whereas coho salmon, jack mackerel, and sablefish performed their feeding activity within the cone of emergence.

On August 30, 1967, coho salmon were observed at two night light stations seaward from Tillamook Head, Oregon (46°09′N lat, 124°45′W long and 45°55′N lat, 124°21′W long). The feeding behavior of coho salmon was identical at the two stations. On each occasion a solitary salmon at the surface within the penumbra would circle the concentration of foraging fishes. To attack, the coho salmon would rapidly charge the concentration of fishes, which churned the surface into a froth, until all observable prey had dispersed. The coho salmon then disappeared but returned and attacked again after the forage fishes regrouped beneath the lights. During the second attack, a hook baited with a live saury was placed in the area of activity. The first station produced a 58-cm coho salmon containing eight saury (9–11 cm long), three blue lanternfish, and a cluster of euphausiids 2 cm in diam. The second station produced a 52-cm coho salmon containing four saury (8–10 cm long), one blue lanternfish, and a few euphausiids. This feeding behavior and stomach content analysis correspond to earlier evidence suggesting that salmon are opportunistic feeders (Reimers, 1964). Prakash (1962) reported that saury was food of coho salmon.

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We observed jack mackerel at three night light stations off the southern Oregon coast (43°52′N lat, 125°10′W long; 43°39′N lat, 124°30′W long; and 41°55′N lat, 125°01′W long) on August 30, 31, and September 1, 1967, repectively. On all occasions they were in schools of up to 40 individuals about 3–5 m beneath the surface, well below the concentrations of saury and lanternfish. When feeding activity began, each jack mackerel swam swiftly toward the surface seeking individuals from the saury–lanternfish concentration. As reported by MacGregor (1966) jack mackerel appeared to select and pursue individual prey. After a few moments, the mackerel would school again for a short time before repeating the feeding pattern. Only one 52-cm FL jack mackerel was caught by hook and line from the lighted surface area. Its stomach contained two saury (10 and 13 cm, FL) and three blue lanternfish. Four additional specimens (49–53 cm, FL) were taken by hook and line about 10–12 m below the vessel. Their stomachs contained primarily lanternfish and paralepidids but no saury.

Sablefish were observed at the following five stations:

Date (1967)	W long	N lat
Aug. 28	46°36′	124°46′
" 29	46°09′	124°45′
" 30	45°55′	124°21′
" 30	45°49′	124°47′
" 31	43°39′	124°30′

They would approach the lighted area either singly or in groups of up to 12 individuals. In all instances, an individual fish would seek out its selected prey at the surface; then leave and wait for the prey to re-concentrate beneath the lights. Their predatory activity appeared to be more premeditated toward single individuals than either of the other species. Several young sablefish were taken by hook and line, midwater trawl, and variable-sized mesh gill nets. Stomachs of 15 fish ranging from 19 to 24 cm, FL, contained predominantly blue lanternfish, euphausiids, and saury (7–11 cm, FL) in that order of abundance. The stomach of one 24-cm male was empty.

The availability of saury relative to its incidence in the stomachs of the observed predators would be a further indication of its elusiveness from slower-swimming predators. The varied stomach contents associated with the available food would suggest that the sablefish and jack mackerel are also species with opportunistic feeding habits.

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Thaw Drip in Frozen American Plaice Fillets, Its Seasonal Variation and Development During Storage

The amount of drip formed on thawing of frozen fish and meat products (thaw drip) varies with a large number of factors (Hamm, 1960; Miyauchi, 1964) involving both freezing and storage conditions and the characteristics and prefreezing condition of the meat sample. Consequently, conditions may combine to give large amounts of thaw drip in a sample of one product and negligible amounts in another. Storage temperature appears to be a major factor (Moran and Hale, 1932; Hamm, 1960) as does holding time prior to freezing (Reay, 1952; Tanaka and Tanaka, 1957) and *p*H (Empey, 1933). But little definitive data on the effect of these factors are available for particular fish products, or of the rate of change of thaw drip during storage.

Frozen flounder fillets have a rather high thaw drip potential, consequently American plaice (*Hippoglossoides platessoides*) was used to investigate the effect on thaw drip of season, of time in commercial storage at about −20 C, and of the effectiveness of brining as a control measure. Fillets were processed from fish obtained approximately monthly from February 1965 to February 1966. Half of each lot was dipped in water as a control, and the other half was dipped in brine (20% saturated) for 15 sec as in commercial practice, prior to freezing (in a commercial plate freezer) in 1-lb (453-g) waxed cartons overwrapped with coated cellophane. Thaw drip, the free drip formed on thawing for 48 hr at 5 C (Dyer et al., 1964), was determined on duplicate 1-lb unwrapped whole blocks thawed without cutting, after frozen storage periods of 1 week, 4, 8, and 12 months. Changes in some other constituents were examined by other investigators, and are reported elsewhere (Ackman, 1967; Castell, unpublished information).

The source of the fish ranged from the Grand Banks to George's Bank (Table I) and was without effect on quality as judged by thaw drip in the limited number of lots available.

Quality prior to freezing, judged by icing times from 3 (2–4) up to 9–10 days, also showed no relation to thaw drip (Fig. 1). In Pacific sole (*Parophrys vetulus*) Good and Stern (1955) observed that time of icing prior to freezing had no effect on thaw drip at icing times greater than 2 days, but an increase occurred during the first 2 days in ice. This initial increase would most likely have already occurred prior to processing of even the freshest samples used in the present work.

The brine dip used was ineffective in reducing thaw drip (Fig. 2 and 3) since differences were not significant at $P = 0.05$. In fish with such high average drip levels, a higher brine concentration in the dip as found for haddock (Holston and Pottinger, 1955) would be necessary to control free drip losses.

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TABLE I. Date of catching, processing date, days in ice on vessel on landing and place where caught, 1965–66.

Catching date	Processing date	Days in ice	Fishing Bank
Feb. 11–13, 1965	Feb. 15	2–4	Banquereau
Mar. 25–26	Apr. 2	7–8	Georges
Apr. 19	Apr. 22	3	Banquereau
May 25 (±5 days)	May 31	1–9	Western
June 16–17	June 22	5–6	Western
July 3	July 9	6	Grand Bank
Aug. 3–4	Aug. 12	8–9	Western or Georges
Oct. 30–31	Nov. 8	9–10	Western
Dec. 18	Dec. 22	4	Banquereau
Jan. 19–20, 1966	Jan. 24	4–5	Western
Feb. 19–20	Feb. 24	4–5	Georges

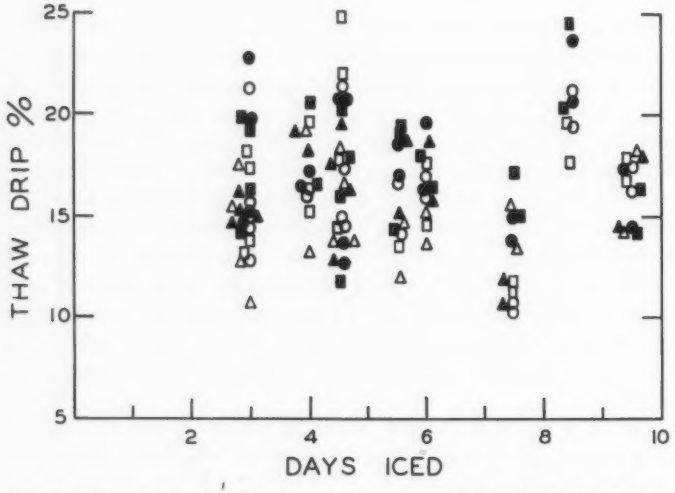

FIG. 1. Icing time (days) on the vessel vs. thaw drip, after 4 (○), 8 (△), and 12 (□) months frozen storage for water-dipped (open symbols) and brined (closed symbols) fillets of American plaice.

Thaw drip increased significantly between 1 week and 4 months of frozen storage.—Although the initial levels of thaw drip in the monthly samples varied considerably, all showed nearly the same relative increase during the time interval between 1 week and 4 months of frozen storage, with little further change at 8 and 12 months. With the exception of the July–August samples, seasonal changes were minor, and results for the other monthly samples are averaged in Fig. 2 for the control and for the brined samples in frozen storage for 1 week and for 4, 8, and 12 months. The levels increase in the

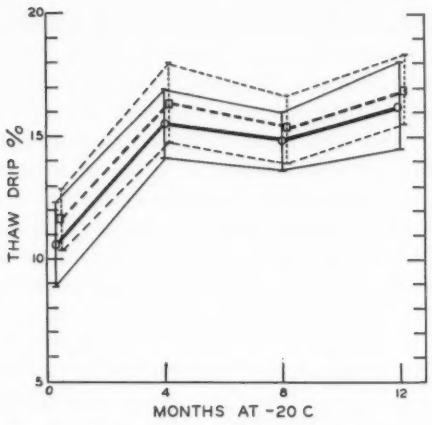

FIG. 2. Thaw drip in water-dipped (——) and in brined (– – – –) fillets of American plaice after 1 week, and 4, 8, and 12 months storage at −20 C. Monthly samples October–June are averaged, 95% confidence intervals of the mean are shown in each case (18 samples each point).

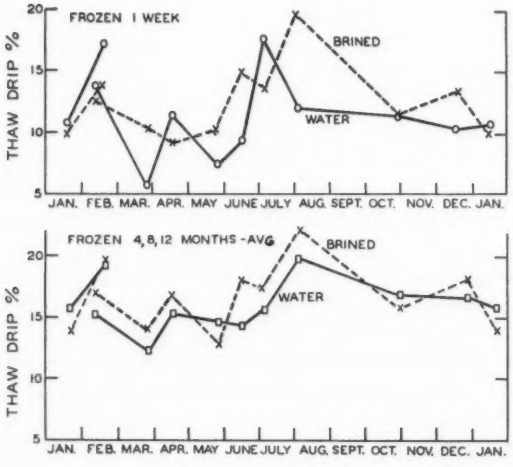
FROZEN 1 WEEK
BRINED
WATER
THAW DRIP %
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN.
FROZEN 4,8,12 MONTHS-AVG
BRINED
WATER
THAW DRIP %
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN.

FIG. 3. Seasonal effect on thaw drip of frozen fillets of American plaice after 1 week (*upper part*), and after 4, 8, and 12 months (averaged in *lower part*) of frozen storage.

control lot from 10.6 at 1 week to 15.5 for the average of the 4-, 8-, and 12-month samples with a standard error of the mean of 0.87 and 0.40 (17 and 54 samples respectively); corresponding values for the brine-dipped samples were 11.6 and 16.5 (SE mean 0.63 and 0.41). Thus increases during frozen

storage were similar in the brined and control fish. During storage no surface dehydration was apparent except in a few of the packages which had been overwrapped with waxed paper (July 3 lot) and in which surface dehydration was manifested by an increasingly thick papery white surface layer. Even at 12 months drying was not sufficient to result in any decrease of thaw drip in these samples, though appearance was poor.

The finding of maximum drip development during the first 2 or 3 months of frozen storage confirms some data on other species. In cod, maximum development is indicated during the first 3 months of storage at −20 and at −10 C (expressible drip — Reay (1952); free drip — Notevarp and Heen, cited in Dyer (1967); Kelly (1967)). It develops in about 3 months in South African hake at −12 C though development in brined and in polyphosphate-treated fish was slower (Pienaar and Thomas, 1964), and after about 2 months in Pacific sole at −18 C (data of Good and Stern, 1955) where the levels reached are similar to those now observed in American plaice. It is thus apparent that although considerable drip development has already taken place on frozen storage for 1 week, as shown by levels of 11% at this time, a large further development, about 40–45%, occurs during the next 2–3 months storage at commercial frozen storage temperatures. Little further change occurs on subsequent storage, however, although if sufficient drying out or "freezer burn" develops, moisture levels and possibly thaw drip will be reduced.

Seasonal effect on thaw drip levels.—A plot of thaw drip values for the samples 1 week in frozen storage shows large variations from month to month with apparent high values in July and August (Fig. 3). Averaged values for 4, 8, and 12 months yield a smoother curve (larger number of samples, 6, for each point) showing an increase from May–June to August followed by a gradual decline to a minimum in the March–May period. More frequent sampling in the July–Oct. period would be desirable to define more fully the nature of the seasonal peak in this species, which is probably related to the post-spawning period of heavy feeding (July–August) and possibly also to variation in size of fish caught at different seasons. The thaw drip peak corresponded to the period of highest lipid level, and greatest susceptibility to oxidation and to disappearance of tocopherol during frozen storage reported on these samples by Ackman (1967), showing that marked seasonal changes occur in the composition of the fish.

No comparable data on seasonal changes in thaw drip appear to exist, though in haddock Crooks and Ritchie (1939) have observed a seasonal increase in moisture content during May and July, and Love (1960) and Dambergs (1964) a similar seasonal effect in cod. Templeman and Andrews (1956) concluded there might be some seasonal variation in moisture content in American plaice, and in addition found thaw drip from commercial pack fillets stored several weeks to several months to range from 9.5 to 27.5% though seasonal variation was not examined.

In conclusion, the high thaw drip losses, averaging 16%, found in American plaice fillets during storage at −20 C indicate that this is an important problem in handling this species. On frozen storage the amount of thaw drip increases about 40–45% in the period between 1 week and 4 months and then remains constant at least to a year. Brining, in a 20% NaCl brine, did not reduce drip significantly. Use of a higher concentration of salt or a polyphosphate dip is indicated, to reduce loss of food constituents, avoid messiness, and to improve yields of thawed product. Special treatment might be necessary to take care of the seasonal very high thaw drip levels found during July and August. Variations in icing time on the vessel, 3–9 days, and of fishing bank, had no apparent effect.

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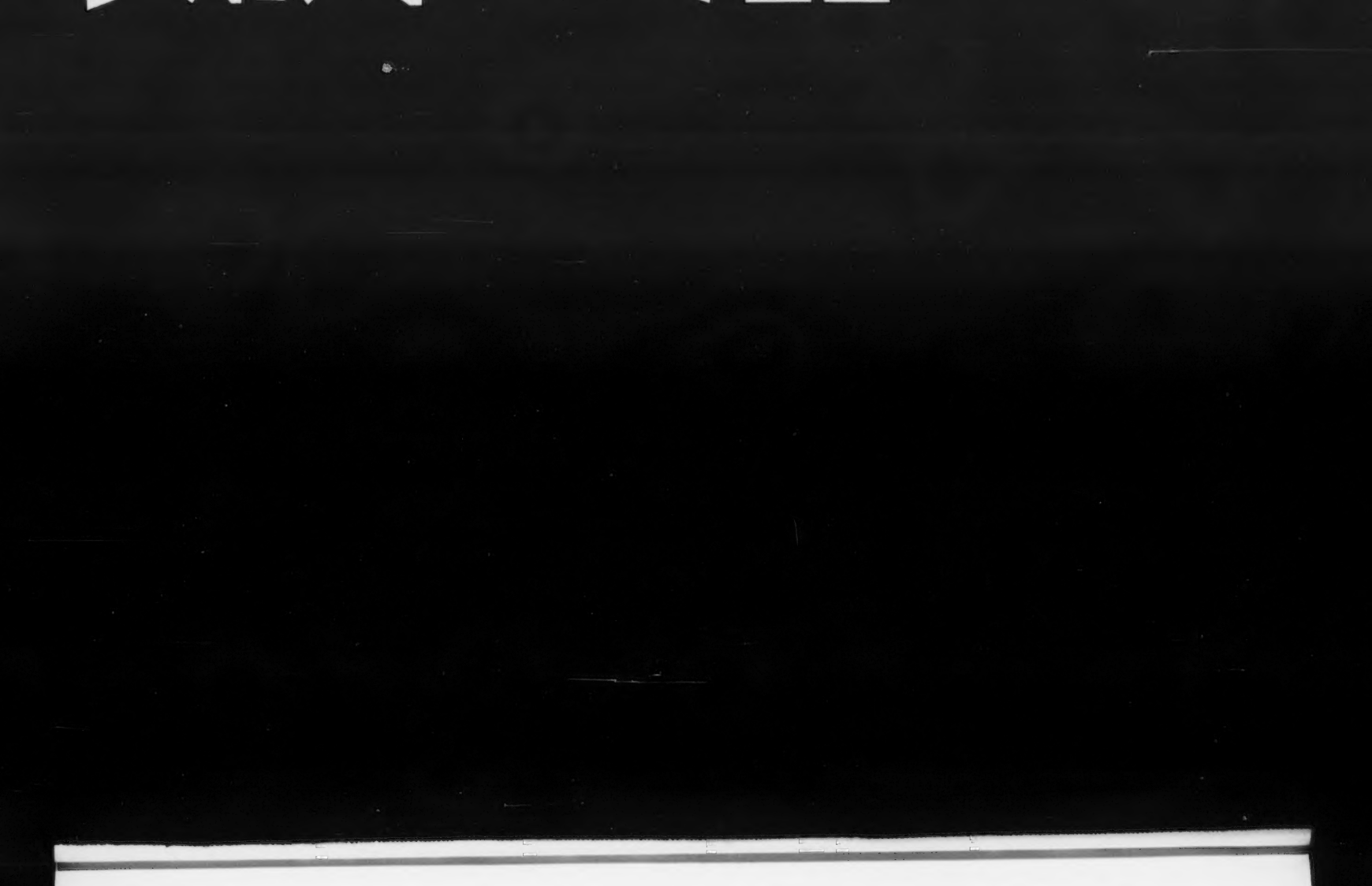
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